# Rice Research for Enhancing Productivity, Profitability and Climate Resilience

H Pathak AK Nayak M Jena ON Singh P Samal SG Sharma



ICAR - National Rice Research Institute Cuttack 753 006, Odisha, India



### RICE RESEARCH FOR ENHANCING PRODUCTIVITY, PROFITABILITY AND CLIMATE RESILIENCE

H Pathak AK Nayak M Jena ON Singh P Samal SG Sharma



ICAR-National Rice Research Institute Cuttack 753 006, Odisha, India



### Citation

Pathak H, Nayak AK, Jena M, Singh ON, Samal P and Sharma SG (2018) Rice Research for Enhancing Productivity, Profitability and Climate Resilience. ICAR-National Rice Research Institute, Cuttack, Odisha, India, p x+542.



Editors H Pathak AK Nayak M Jena ON Singh P Samal SG Sharma

#### Published by

Director, ICAR-National Rice Research Institute, Cuttack 753 006, Odisha, India

**Design and layout** SK Sinha

February, 2018

Disclaimer

ICAR-National Rice Research Institute, Cuttack, Odisha is not liable for any loss arising due to improper interpretation of the scientific information provided in this book.

©All Rights Reserved ICAR-National Rice Research Institute, Cuttack, Odisha, India

Laser typeset at the ICAR-National Rice Research Institute, Cuttack 753 006, Odisha, India. Printed by the Print-Tech Offset Pvt. Ltd., Bhubaneswar 751 024, Odisha, India for Director, ICAR-National Rice Research Institute, Cuttack 753 006.





त्रिलोचन महापात्र, पीएच.डी. एफ एन ए. एफ एन ए एस सी. एफ एन ए एएस सचिव एवं महानिदेशक

TRILOCHAN MOHAPATRA, Ph.D. FNA, FNASC, FNAAS SECRETARY & DIRECTOR GENERAL भारत सरकार कृषि अनुसंधान और शिक्षा विभाग एवं भारतीय कृषि अनुसंधान परिषद कृषि एवं किसान कल्याण मंत्रालय, कृषि भवन, नई दिल्ली 110 001 GOVERNMENT OF INDIA DEPARTMENT OF AGRICULTURAL RESEARCH & EDUCATION AND INDIAN COUNCIL OF AGRICULTURAL RESEARCH MINISTRY OF AGRICULTURE AND FARMERS WELFARE KRISHI BHAVAN, NEW DELHI 110 001 Tel: 23382629: 23386711 Fax: 91-11-23384773 E-mail: dgi.car@nic.in

### FOREWORD

Rice, a major staple crop, feeds more than half of the world population, employs millions of people and has enormous impacts on environment. Globally, the crop is planted in about 160 million hectare with production of 493 million tons of milled rice. In India, rice plays a major role in diet, economy, employment, culture and history. It is the staple food for more than 65% of Indian population contributing about 40% to the total food grain production, thus playing a pivotal role in the food and livelihood security of people.

Increase in productivity and profitability of rice on a sustainable basis is possible through novel technological inputs that can address various challenges, including those arising from climate change. Scientific advances in high throughput genotyping-phenotyping and genomics—assisted breeding provide opportunities to explore and embed genes for improving yield and quality. Cutting-edge research enables opportunity to break the yield ceiling and breed new generations of climate-ready rice varieties. Management of soil, nutrient and water has assumed greater significance with the emerging changes in economic and environmental scenario. Improvements in sensors, processing, communications, and nanotechnology offer the potential to increase the use efficiencies of water, fertilizer and plant protection chemicals.

Concerted efforts of agricultural scientists and rice farmers supported by Government policies ensured production of 110 million tons of milled rice during 2015-16. This enabled us in meeting the domestic demand and allowed export of rice. However, to ensure food and nutritional security for the ever-growing population in the face of impending adversities due to climate change, the efforts to increase rice production have to be sustained.

This book on "**Rice Research for Enhancing Productivity, Profitability and Climate Resilience**" published by ICAR-National Rice Research Institute, Cuttack, Odisha analyzes the major emerging problems, reviews the achievements and identifies research and development needs for sustainable rice farming. The chapters comprehensively cover various aspects of crop improvement, production, protection, physiology, biochemistry, economics and extension of rice technologies. Exclusive information on enhancing productivity, profitability and climate resilience in the context of global as well as Indian scenarios has been captured in the book.

I hope that the book will help in understanding the emerging challenges to rice farming. I congratulate the editors and authors for their contributions in bringing out this useful publication that would serve as a reference book on rice farming for researchers, students, policy makers and civil society.

Mugnt-(T. MOHAPATRA)

Dated the 7<sup>th</sup> February, 2018 New Delhi

### Preface

Rice, the world's most important food crop, has been grown for more than 6000 years in South Asia. Wild rice, however, grew in the Himalayas 15,000-16,000 years back. Currently, rice is the staple food for about four billion people i.e., half of the humankind on the planet. Rice fields cover around 160 million hectares in a wide range of climatic conditions spanning from 44°N in North Korea to 35°S in Australia. It is cultivated from 6 feet below sea level (such as in Kerala, India) to 2700 feet above sea level in the Himalayas. The crop occupies a significant position in the culture and heritage of many Asian countries. In India, particularly in the eastern states, it is a part of almost every ritual. The crop has been referred in the Vedas, Ramayana, Mahabharata, Buddhist and other ancient literature.

India has the world's largest area and is the second highest producer of rice. The crop is grown under varying climatic and soil conditions under diverse ecologies spread over about 43 million hectares. The crop is cultivated round the year in one or the other parts of the country. Since last two years the country has recorded the highest rice production of about 110 million tons. It is the staple food for more than two thirds of Indian population contributing more than 40% to the total food grain production, thereby, occupies a pivotal role in the food and livelihood security of people.

During the last decades, significant advancements have been made on developing high yielding and disease-resistant rice varieties and production technologies for different ecologies. The country, so far has released about 1200 varieties. Several viable rice production technologies have also been developed for adoption in the farmers' fields. Along with increasing the productivity, emphasis has been given on developing varieties with improved stress-tolerance and nutritional quality to ensure nutritional security for the large section of the population depending on rice as staple food. Currently, about 85% of rice area is covered with high-yielding varieties. India's rice export has also steadily increased with the current export of more than 10 million tons annually making it the leading rice exporter.

However, in the backdrop of all these achievements, *rice farmers and researchers are facing new challenges of* climate change, low water availability, poor soil health, low nutrient use efficiency and increased emergence of insects and diseases. There is now growing concern that non-price factors such as declining scope for further gains from existing modern varieties, deteriorating soil and ground water supplies, and reduced public investment in research have contributed to poor productivity growth in recent years. The challenge is to integrate productivity and profitability improvement of rice while enhancing the climate resilience and quality of the environment on which production depends.

The objective of the publication 'Rice Research for Enhancing Productivity, Profitability and Climate Resilience', published by ICAR-National Rice Research Institute, Cuttack, Odisha is to review the scientific and technical literature available on the developments in rice science and its implications on productivity, profitability and climate resilience; provide science-backed, policy-relevant information for improving rice farming and suggest implementable research and development guidelines to the stakeholders.

Thirty-one chapters of the book cover the state-of the-art information in national and international contexts on enhancing productivity, profitability and climate resilience. The chapters capture various aspects of rice viz., (1) improvement i.e., conservation of genetic resources; quality seed production; genetic improvement for enhancing resistance to biotic stresses, input use efficiency, aroma, nutrition and grain quality, and climate resilience; harnessing heterosis; new generation rice for breaking yield ceiling and biotechnological strategies and genomic resources for rice improvement; (2) production i.e., enhancing productivity and resource use efficiency; decreasing energy and water footprints; agro-ecology-based intensification; integrated rice-based farming systems; resource conserving technologies; weed management; efficient use of rice straw; mechanization of rice-based cropping systems and harnessing microbial resources; (3) protection i.e., exploration of new sources of resistance; bio-ecology of rice pest and diseases; bio-intensive approaches and optimization of chemical pesticide-use for management of rice pest; (4) physiology and biochemistry in relation to grain quality and nutritional improvement; multiple abiotic stress tolerance and improvement of photosynthetic efficiency of rice; and (5) socio-economic aspects i.e., vield gap analysis and impact assessment to aid rice research and policies, and developing extension approaches to enhance income of rice farmers. The chapters comprehensively present the technologies for effective management of rice crop in favorable and unfavorable ecologies to make rice farming profitable and sustainable. The authors have also captured the achievements of the ICAR-National Rice Research Institute on rice research and development over the years.

In the course of preparing the book, the authors and editors have received help and support from different individuals. We are extremely grateful to each one of them. The editors take this opportunity to express their gratitude to all the authors for developing the chapters in a comprehensive and time-bound manner. We thank Dr. T. Mohapatra, Director General, Indian Council of Agricultural Research and Secretary, Department of Agricultural Research and Education for taking keen interest in bringing out this publication. We are thankful to the members of the publication committee of NRRI for their help and support and Mr. Sunil Sinha for type-setting, formatting and developing the outline, cover page of the publication.

We hope that the publication would be useful to the researchers, teachers, policy makers, planners, administrators, progressive farmers and students of rice sciences.

H Pathak AK Nayak Mayabini Jena ON Singh P Samal SG Sharma

#### **EDITORS**

### Content

Sl. No.	Topic Page
1.	Foreword
2.	Preface
3.	Revitalizing Rice-System for Enhancing
4.	Rice Genetic Resources: Collection, Conservation,
5.	Quality Seed Production and Maintenance Breeding for
6.	Utilization of Cultivated and Wild Gene Pools of Rice for
7.	Enhancing Input Use Efficiency in Direct-seeded Rice with
8.	Genetic Improvement of Rice for Aroma, Nutrition and
9.	Genetic Improvement of Rainfed Shallow-lowland Rice for 107 Higher Yield and Climate Resilience SK Pradhan, M Chakraborti, K Chakraborty, L Behera, J Meher, HN Subudhi, SK Mishra, E Pandit and JN Reddy
10.	Genetic Improvement of Rice for Multiple Stress

Sl. No.	Topic Page
11.	Harnessing Heterosis in Rice for Enhancing
12.	New Generation Rice for Breaking Yield Ceiling
13.	Biotechnology for Rice Improvement: Achievements and
14.	Development of Genomic Resources for Rice Improvement
15.	Nutrient Management for Enhancing Productivity and
16.	Assessing Energy and Water Footprints for
17.	Agro Ecological Intensification of Rice-based Cropping
18.	Integrated Rice-based Farming Systems for Enhancing

Sl. No.	Topic Pag	;e
19.	Resource Conservation Technologies under Rice-based	0
20.	Dynamics and Management of Weeds in Rice	Π
21.	Economic and Eco-friendly Use of Rice Straw	2
22.	Rice Mechanization in India: Key to Enhance Productivity	9
23.	Microbial Resources for Alleviating Abiotic and Biotic	4
24.	Exploring New Sources of Resistance for Insect Pest and	0
25.	Bio-ecology of Rice Insect Pests and Diseases: Paving the	4
26.	Bio-intensive Management of Pest and Diseases of Rice	4
27.	Optimization of Chemical Pesticide use in Rice	9

Sl. No.	Topic Page
28.	Improving Protein Content, Glycemic Index, Mineral
29.	Improvement of Photosynthetic Efficiency of Rice:
30.	Abiotic Stress Tolerance in Rice: Physiological Paradigm
31.	Innovative Extension Approaches for Increasing Income
32.	Quantification of Yield Gaps and Impact Assessment of Rice
33.	Climate Resilient Production Technologies for Rainfed
34.	Acronyms
35.	Editors and Authors



### Revitalizing Rice-Systems for Enhancing Productivity, Profitability and Climate Resilience

### H Pathak, P Samal and M Shahid

### SUMMARY

Rice (Oryza sativa L.) is the staple food for about half of the global population, grown in 160 million ha (Mha) with 493 million tons (Mt) milled rice production. Out of about 141 Mha of net cultivated area in India, rice occupies the maximum i.e., about 43 Mha. Over the last four decades, rice production has witnessed impressive growth due to the development of high-yielding varieties, coupled with the adoption of intensive input-based management practices. The task of increasing rice production has become quite challenging, however, in recent years due to degradation in natural resources such as soil, water and air along with shortage of labour and emerging problem of climate change. In future, rice would have to be produced using less land, water and labour through more efficient, environment-friendly production systems that are more resilient to climate change and also contribute less to greenhouse gas emission. Moreover, all of our efforts so far were production-centric. Now we need to shift the focus to make it profit-centric. In the process of pursuing higher yield, we neglected the environment i.e., soil, water and air, considering them to be passive and inactive players of crop production. To increase productivity, profitability, climate resilience and sustainability of rice production, a range of strategies i.e., technological, infrastructural and policy need to be adopted to transform the current productiondriven rice-based cropping system to profit-driven rice-based farming system. Agricultural research should be re-oriented with farmers' participatory approach to unshackle the vicious circle of poverty, reduce drudgery and fulfill the aspirations of resource-poor, smallholder rice farmers.

### **1. INTRODUCTION**

Rice is the staple food for about half of the world population (Table 1). Grown for more than 6000 years, it is economically, socially, and culturally important to a large number of people across the globe. More than 100 countries grow rice with the third highest worldwide production of 740 million tons (Mt) of rough rice, after sugarcane and maize. It accounts for 35-75% of the calories for more than 3 billion Asians. Globally, it provides 27% of dietary energy, 20% of dietary protein and 3% of dietary fat. Rice fields cover around 160 million hectares, the third largest cereal, and most important food of majority of global poor. It is grown in a wide range of climatic conditions spanning from 44°N latitude in North Korea to 35°S latitude in Australia. It is cultivated from 6 ft below sea level (such as in Kerala, India) to 2700 ft above sea level. Most of the rice in tropical countries is produced in irrigated and rainfed lowland areas. Irrigated rice systems account for 78% of all rice production and 55% of total



Global scenario		Indian sc	enario
Magnitude	Per cent <sup>a</sup>	Magnitude	Per cent <sup>b</sup>
4	56	0.8	65
144	25	67	56
160	10	43	22
493	20°	110	30°
2.95	80 <sup>d</sup>	2.56	82 <sup>d</sup>
400	40 <sup>e</sup>	150	40 <sup>e</sup>
206	13	53	17
25	15	6	25
880	35	200	30
25	12	3.5	18
	Magnitude           4           144           160           493           2.95           400           206           25           880	Magnitude         Per cent <sup>a</sup> 4         56           144         25           160         10           493         20 <sup>e</sup> 2.95         80 <sup>d</sup> 400         40 <sup>e</sup> 206         13           25         15           880         35	MagnitudePer cent <sup>a</sup> Magnitude4560.81442567160104349320°1102.9580 <sup>d</sup> 2.5640040°15020613532515688035200

### Table 1. Global and national importance of rice.

Note: Data pertain to 2015-16. a, per cent of global total; b, per cent of country total; c, of total food production; d, of agriculture; e, of rural poor

Source: Compiled from various publications from IRRI (2016); NRRI (2016), MoA (2016)

harvested rice area, mostly concentrated in alluvial floodplains, terraces, inland valleys, and deltas in the humid and sub-humid subtropics and humid tropics of Asia. The crop occupies largest area in India followed by China and Indonesia, whereas China has the highest production but, Australia has the highest productivity (Table 2).

	Area		Production		Yield
Country	(Mha)	Country	(Mt)	Country	(t ha <sup>-1</sup> )
India	43.67	China	137.64	Australia	6.68
China	30.67	India	105.79	Egypt	6.37
Indonesia	13.69	Indonesia	46.93	USA	5.66
Bangladesh	11.67	Bangladesh	34.27	Spain	5.14
Thailand	11.49	Vietnam	29.50	Turkey	5.11

Table 2. Area, production and yield-wise top five countries (2012-14).

Source: FAOSTAT (2016)

In India, rice plays a major role in diet, economy, employment, culture and history. It is the staple food for more than 65% of Indian population contributing approximately 40% to the total food grain production, thereby, occupying a pivotal role in the food and livelihood security of people. The country has the world's largest area under rice i.e., about 43 million hectare (Mha) and the second highest production i.e., about 110 Mt of milled rice at productivity of 2.56 t ha<sup>-1</sup> (Table 1) as per 2016-17 statistics. The crop is cultivated round the year in one or the other parts of the country. The leading rice producing states are West Bengal, Uttar Pradesh, Punjab, Odisha, Andhra Pradesh, Bihar and Chhattisgarh (Table 3). About 40% of the rice area in India is rainfed and more than 70% of which is in eastern India. Out of the total rainfed area, 23% are rainfed upland and 77% are rainfed lowland. The entire rainfed upland and 52% rainfed lowlands are drought prone. About 17% of rainfed lowlands are flood prone.



Table 3. Area, production and productivity of rice in different states of India (2015-	
16).	

State	Area (000 ha)	Production (000 ton)	Yield (t ha-1)
Uttar Pradesh	5862	12501	2.13
West Bengal	5524	15954	2.89
Odisha	3941	5875	1.50
Chhattisgarh	3816	5789	1.51
Bihar	3232	6802	2.10
Punjab	2975	11823	3.97
Assam	2485	5125	2.06
Andhra Pradesh	2161	7489	3.47
Madhya Pradesh	2024	3547	1.75
Tamil Nadu	2000	7517	3.76
Jharkhand	1589	2882	1.81
Maharashtra	1503	2593	1.72
Haryana	1354	4145	3.06
Karnataka	1110	3021	2.72
Telangana	1046	3047	2.91
Others	2878	6298	2.19
India	43500	104408	2.40

Source: Ministry of Agriculture, Government of India.

Global demand of rice needs to increase from the current 493 Mt to about 550 Mt in 2030 (IRRI, 2016). However, rice farming, particularly in the rainfed regions, faces multiple risks from uncertain climate, degraded soil, water shortage and underdeveloped markets. It has come under increasing pressure from intense competition for land and water, a more difficult growing environment because of climate change, higher price for energy and fertilizers, labour shortage, increasing cost of cultivation, declining profit margin and greater demand for reduced environmental footprint (Samal 2009; Samal 2013). The socio-economic dynamics and food habits are also changing adding another dimension to already complex challenges of rice cultivation. Therefore, the goal of rice research and development should be at improving nutritional and income security of rice farmers while addressing environmental sustainability and coping with climate change.

The chapter deals with the rice ecosystems; trends in area, production and productivity; emerging challenges and the strategies for enhancing productivity and profitability of rice production in India.

### 2. RICE ECOSYSTEMS IN INDIA

In India, rice is grown under highly diverse conditions with area stretching from  $79^{\circ}$  to  $90^{\circ}$ E longitude and  $16^{\circ}$  to  $28^{\circ}$ N latitude under varying agro-ecological zones. It is cultivated mostly in wet season with unpredictable rainfall distribution. It is also



grown in areas, where water depth reaches 2-3 m or more. Rice culture in Kuttanad district of Kerala is grown below the sea level, while in the state of Jammu and Kashmir, it is grown upto an altitude of 2000 m above sea level; with temperature range of 15-40 °C and average annual rainfall range from 30 mm in Rajasthan to more than 2800 mm in Assam. A wide range of rainfall distribution pattern (drought, submergence, deep water) and distinct differences in soils (coastal and inland salinity, alkalinity, acidity), agro-climatic situations (high humidity) and seasons has resulted in the cultivation of thousands of varieties and therefore, one can see a standing rice crop at some parts of the country or the other in any time of the year. Rice is primarily grown under four major ecosystems broadly classified as (i) irrigated, (ii) rainfed lowland, (iii) rainfed upland and (iv) flood prone.

- Irrigated rice eco-system: Total area under irrigated rice in the country is about 26.0 Mha accounting for about 60% of the total area under the crop. It includes the areas in Punjab, Haryana, Uttar Pradesh, Jammu & Kashmir, Andhra Pradesh, Telangana, Tamil Nadu, Karnataka, Himachal Pradesh and Gujarat.
- Rainfed lowland rice ecosystem: In India, lowland rice covers an area of about 14.0 Mha, which accounts for about 32% of the total area, located mainly in eastern India. The area is characterized by poor soil quality and frequent occurrence of drought/flood due to erratic rains.
- Rainfed upland rice ecosystem: Total area under rainfed upland rice in the country is about 6.0 Mha, which accounts for 13.5% of total area, located mainly in eastern zone i.e., Assam, Bihar, Chhattisgarh, eastern Uttar Pradesh, Jharkhand, Madhya Pradesh, Odisha, West Bengal, and North East Hill Region. The rainfed upland ecosystem is drought prone.
- Flood-prone rice ecosystem: It occupies about 2.5 Mha in eastern states of the country. The crop is grown in shallow (up to 30 cm), semi-deep (30-100 cm) and deep-water (>100 cm) ecosystems in eastern Uttar Pradesh, Bihar, West Bengal, Assam and Odisha.

Among the above ecosystems, further sub-systems are usually identified for location-specific variations such as 'favourable' or 'unfavourable' moisture, soil type, temperature regime, proneness to drought, submergence, both drought and submergence; growth duration (early, medium, late maturity groups) and low light intensity conditions.

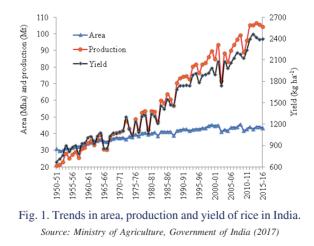
### **3. ACHIEVEMENTS OF RICE RESEARCH**

During the last five decades, a lot of advancements have been made on developing high yielding and disease-resistant varieties and production technologies for different ecosystems. The country has released about 1200 varieties including about 240 varieties released by ICAR so far for different ecologies. Most of the recent releases are resistant to multiple diseases such as blast, sheath blight, sheath rot, false smut, brown spot, stem rot and bacterial blight. These varieties are also tolerant to different



pests (gall midge, brown plant hopper, stem borer). Many varieties among them have early maturity duration. Several viable rice production technologies have also been developed for adoption in the farmers' fields. Besides, the duration of maturation of aromatic rice has been reduced from 160 days to 110 days, while the yields have increased from less than 2.5 t ha<sup>-1</sup> to more than 7.0 t ha<sup>-1</sup>. Shortening of the crop cycle has not only helped in saving water and labour, but also facilitate new combinations of crops in rotations. The hybrid rice technology contributed towards an additional 4-5 Mt to the total rice production in the country and there is a vast scope for increased adoption of this technology by the farming community in future. Along with increasing the productivity, emphasis has been given on improving the nutritional quality of rice varieties and developed varieties with improved quality attributes such as high protein (CR Dhan 310, CR Dhan 311), high zinc (DRR Dhan 45), low glycemic index (Improved Sambha Mahsuri) in rice to provide nutritional security to the population depending on rice as staple food. High vielding varieties of rice developed and released by ICAR-institutes and SAUs have reached millions of farmers in different states of the country and are cultivated under different agro-ecologies. Currently, about 85% of rice area is covered with high-yielding varieties. Stress tolerant varieties have also helped steady production levels making rice production systems climateresilient. India's rice export has also steadily increased making it the leading rice exporter followed by Thailand, Vietnam, USA and Pakistan. Currently, the country exports about 10 Mt rice annually.

Production of rice has increased more than five times since 1950-51 and made India self-reliant in rice from early 1980s (Fig. 1). The sources of growth in the past were increase in area and yield, which has increased by 1.4 and 3.6 times, respectively since 1950-51. Though during Green Revolution period the production growth has accelerated, during 2000s, the growth has decelerated, threatening the national food security. It is observed that the additional production during 2000s has decreased over the previous decade. More precisely, additional production during 1990s over





1980s was 20.3 million tons, which reduced to 9.2 tons during the 2000s, indicating thereby that the production base is shrinking, which is a cause of concern. The additional yield has also reduced from 387 kg ha<sup>-1</sup> during 1990s to 200 kg ha<sup>-1</sup> during 2000s. However, some signs of improvement were noticed during the recent period (2010-11 to 2015-16) and the additional average yield has increased from 200 kg ha<sup>-1</sup> during 2000s to 332 kg ha<sup>-1</sup> during 2010-16. A major share of production increase during the recent period is from the eastern states (Assam, Bihar, Chhattisgarh, Jharkhand, Madhya Pradesh, Odisha, Uttar Pradesh and West Bengal). It is also observed that the area growth has almost exhausted in rice. These observation leads to the conclusion that the area growth in India has been exhausted and further increase in production has to come from yield increase only.

### 4. PROJECTED DEMAND OF RICE

Population growth is the major driving force for increasing rice demand in India. In addition, the low-income segment of the population will also demand more rice with increase in income. It is estimated that about 120 and 140 Mt of rice would be required by 2025 and 2050, respectively in India. In addition, India is exporting about 10 Mt of rice per year, which earns valuable foreign exchange for the country. There is a growing middle-class, rice-consuming population in domestic as well as international markets. This will increase the demand for high-quality rice creating great opportunities for India for exporting basmati and high-quality non-basmati rice. This increased production has to necessarily come from increased productivity rather than increase in area under rice and that too under deteriorating soil, water and other natural resources. Therefore, to sustain present food self-sufficiency and to meet future demand of food and export, the production has to increase by about 1.5 Mt yr <sup>1</sup> and the productivity to 3.25 t ha<sup>-1</sup> by 2050 from the current level of 2.56 t ha<sup>-1</sup> (2016-17) i.e., an increase of about 30%.

### 5. EMERGING CHALLENGES OF RICE FARMING

Rice production is intricately linked with land and water, and this has unique and profound implications for the environment. Hence, careful management of the natural functioning of rice ecosystems is critically important for protecting the environment while raising rice productivity to meet growing demand. The Green Revolution in south Asia in the 1960s helped doubling food production with a mere expansion in area of 10–20% and introducing seeds of high-yielding varieties, fertilizers, irrigation, and pesticides. However, this intensification of agriculture had adverse environmental consequences such as the deterioration of natural resources. As a result, the increase in productivity is showing signs of slowing down/stagnating. Yield trends from long-term continuous rice-rice experiments conducted in Bangladesh, China, India, Indonesia, Nepal, the Philippines, and Thailand indicated that, even with the best available cultivars and scientific management, rice yields (holding input levels



constant) have either stagnated or declined over time since the early 1980s. The ricewheat cropping system of the Indo-Gangetic Plains (IGP) also showed yield stagnation/ decline in the last two decades. Earlier gains in rice output were driven by the increase in area under modern varieties, irrigated area, fertilizer use, increased cropping intensity, and supportive input and output price policies in irrigated areas. However, in the last decades, there is sign of yield plateauing in major irrigated states. There is now growing concern that other non-price factors, such as the declining scope for further gains from existing modern varieties, deteriorating soils and ground water supplies, and reduced public investment in research have contributed to poor performance in recent years in irrigated areas. The challenge is to integrate productivity and profitability improvement while conserving and enhancing the quality of the environment on which production depends. The major environmental concerns of modern rice farming and suggested potential remedial measures are presented below (Pathak and Ladha 2006).

### 5.1. Degrading the water resource base

The productivity of water for rice is very low. Rice requires about two times as much water as wheat or maize. In some regions, such as northwest India, water application in rice is about 5-6 times more than that of wheat. Large water demand of rice is expected to outstrip the available supply in the near future. The declining availability and quality of water, increased competition from domestic and industrial sectors, and increasing costs are already affecting the sustainability of irrigated rice production systems in many parts of south Asia. For example, in the upper transect of the IGP, rice cultivation resulted in a decline in water tables and water quality. A rapidly depleting water table in many northern and southern states is also a matter of concern for future productivity growth. Five major rice surplus states, Punjab, Haryana, western Uttar Pradesh, Tamil Nadu, and erstwhile Andhra Pradesh, where groundwater depletion is a major issue, account for around 42% of India's rice production (in 2015-16). These states contribute a significant amount to the country's central pool, which is critical for India's food security and also to the functioning of India's food distribution program, through which poor people are provided with highly subsidized grains. Many districts in the rice-wheat growing area of Haryana, and Punjab, show a water table decline in the range of 3-10 m over the last two decades. The groundwater table has fallen at about 23 cm yr<sup>-1</sup> in the central Punjab. India. The other side of the water problem is waterlogging in some areas. In some districts of Haryana, the water table is rising at 0.14 to 1.0 m yr<sup>-1</sup> and more than 0.4 Mha of land has a water table within 3 m of the soil surface. Apart from water scarcity, the growing demand for land from urbanization, industrialization, and for growing cash crops is likely to cause a decline in rice area. According to the NRRI 2050 vision document, rice area may decline by 6-7 Mha by 2050, a decline of around 15% in the next 35 years. In other words, India will need to produce 137 million tons of rice on 37 Mha of land in 2050 compared with the current production of 105 Mt of rice on 43 Mha. Therefore, yield will have to increase by 50% in the next three decades to keep India food secure.



The demand for fresh water is growing from other sectors of the economy like industry, domestic and environmental use besides water for irrigation purpose to raise crops. Among cereals, rice consumes much more water than others and it is estimated that 2500-5000 liters of water is required to produce one kg of rice. As the demand for water by all the sectors of the economy grows, ground water is being depleted, water reservoirs and canals is being silted, other water ecosystems are becoming polluted and degraded, and developing new sources of water is getting more costly, policy makers and researchers are concerned that water will be the main obstacle for growing enough food in the coming years.

Water application in rice production needs to be decreased by increased wateruse efficiency through reduced losses caused by seepage, percolation, and evaporation; laser land leveling; crack plowing to reduce bypass flow; and bund maintenance. Management options to increase the efficient use of rainwater include crop scheduling, diversified cropping, and the construction of small ponds serving as on-farm reservoirs for water harvesting. Various crop and water management systems such as water-saving irrigation techniques, intermittent drying of the soil, growing rice with reduced or no tillage either on flat land or raised beds, and shifting away from continuously flooded (anaerobic) to partly or even completely aerobic rice can drastically improve the efficiency of water use.

### 5.2. Degrading soil resource base

Concerns about sustainability are arising throughout tropical rice ecosystems because of decreasing soil fertility as most countries move into the post-Green Revolution era. Recent trends of yield decline/stagnation observed in long-term experiments in south Asia were mostly due to soil-related causes such as the decline in soil C and macro- and micro-nutrients in rice-rice and rice-wheat systems; accumulation of phenolic compounds, Fe<sup>2+</sup>, and sulfides in the rice-rice system; and the increase in soil salinity. Intensive use of irrigation water in rice led to a salinity buildup. In Pakistan's Sindh Province, large areas became saline after the introduction of extensive irrigation. In the short term, salinity buildup leads to reduced yields, whereas, in the long term, it can lead to abandoning of crop lands. Farmers are also using poor-quality water for irrigation in several areas of the Indo Gangetic Plains for rice and run the risk of further aggravating soil degradation. The soil quality of rice systems therefore, needs to be continuously monitored.

Though farmers apply some of the macro-nutrients like N, P and K, they usually neglect application of micronutrients. Even the N, P, K fertilizers are not applied proportionately. In the long run, this causes an imbalance in soil and plant nutrition resulting in yield decline. It has been reported that during 1950s, there was only one nutrient deficiency (Nitrogen), which has increased to eight (N, Fe, P, Zn, K, S, Mn, B, Mo) during 1990s. Moreover, the soil quality is deteriorating from the loss of organic carbon, erosion, soil compaction, salinization, heavy metal introgression into soil from industries and pesticides, and other anthropogenic activities.

### 5.3. Burning of rice residues

Rice straws and husks are often not disposed of in an environment-friendly manner. In a recent survey, it was noted that 60% and 82% of rice straw produced in the northwestern states of Haryana and Punjab, respectively, is burned in the field (Pathak et al. 2012). About 20 Mt of rice residues are burned annually in Punjab, India, alone. The burning of rice straw is environmentally unacceptable as it leads to (1) the release of soot particles and smoke, causing human health problems such as asthma or other respiratory problems; (2) emission of greenhouse gases such as carbon dioxide, methane, and nitrous oxide, causing global warming; and (3) loss of plant nutrients such as N, P, K, and S. Almost the entire amounts of C and N, 25% of P, 50% of S, and 20% of K present in straw are lost due to burning.

One potential solution to the problem of rice straw burning would be its retention on the soil surface. This straw mulch reduces moisture loss from soil, controls weeds, manipulates soil temperature for better crop growth, and improves soil organic matter content. With the development of new machines (such as the Happy Seeder), it is now possible to sow seeds in residue-retained fields.

### 5.4. Climate change

Climate change effects include increase in temperature in the long-run, changes in rainfall regimes with increasing year-to-year variability and a greater prevalence of extreme events. Climate has changed worldwide over the last century. It is predicted that a warming of 5°C can eliminate 20% of the coastal wetlands by the year 2080. Apart from rice area reduction, rice yield under simulated global climatic change scenarios have shown a decline, when temperature increase is more than  $0.8^{\circ}$ C per decade. It is feared that a 20% decline in rice yields can occur in north-west India due to elevated CO<sub>2</sub> and temperature. At present, we do not have thermo-insensitive rice varieties with comparable yield levels within the present high yielding varieties to cope up with the situation. Besides the above, emergence of new pests with higher degree of virulence will be there due to changing climate.

Rice production contributes to global climate change (through emissions of methane and nitrous oxide) and in turn suffers from the consequences. An increase in temperature has two effects on rice: decreasing spikelet fertility due to higher maximum temperature and increasing respiration due to higher minimum temperature. The increase in temperature, especially that of mean minimum night-time temperature, has adverse effects on rice productivity as it reduces crop duration, increases respiration of pest populations, hastens nutrient mineralization in soils, decreases fertilizer-use efficiencies, and increases evapo-transpiration (Wassmann et al. 2009a; 2009b). An increase in atmospheric carbon dioxide, on the other hand, has a fertilization effect on rice, promoting its growth and productivity. Recent studies, however, suggested that the effect of global warming would be largely negative for rice production because of increased respiration and a shortened vegetative and grain-filling period. It is believed



that climate change would affect the quality of crops, particularly important aromatic crop such as basmati rice. In addition to direct effects on rice plants, climate change and global warming might affect other organisms associated with rice and thus, alter the occurrence and severity of rice pests. There is also a need to meet the challenge of the increase in extreme climatic events associated with climate change, such as the increasing severity and frequency of floods and droughts, as well as more frequent hurricanes, and their effects on rice production.

Field measurements in several Asian countries have identified possible technical options to mitigate methane emissions through modified water regime (mid-season drainage, alternate flooding), modified residue management (sequestration of straw), use of additives (phosphogypsum, nitrification inhibitors), and modified land management (direct seeding, reduced tillage and site-specific nutrient management) (Pathak 2015). Similarly, novel approaches of demand-driven N supply using leaf color charts and site-specific N management minimize the pool of excessive nitrogen in the soil and thus reduce nitrous oxide emissions. At the same time, adaptation strategies such as (1) resilient varieties for moisture-stress environments, (2) management systems that reduce water use, and (3) insect-pest and disease resistant varieties should be developed to overcome the ill effects of climate change and climatic variability.

### 5.5. Loss of biodiversity

The introduction of modern rice varieties and practice of monoculture have caused a reduction in and loss of biodiversity as many traditional varieties have been abandoned when farmers found modern varieties to be more productive and profitable. Genetic diversity is required for the continual improvement of the rice crop, as cultivars need to be invigorated every 5 to 15 years to better protect them against diseases and insect pests. With the advances in biotechnology, there is a need for a diversity of genetic material for the potential of these technologies to be fully achieved.

### 5.6. Increasing cost of cultivation and decreasing farmers income

The cost of cultivation has increased and profits decreased over years in majority of the states. The cost of cultivation per ha across states varied from Rs. 37,071 to Rs. 78,968 and profits were either low or negative in many rice-growing states (Table 4). Policy makers are now concerned, how to make rice cultivation remunerative and at the same time supply consumers rice at affordable prices.

### 5.7. Increasing labour shortage

With the process of development, the non-farm sector is growing and young people are attracted to non-farm jobs due to higher wage rates in that sector and higher drudgery involved in agricultural operations. Therefore, growing labour shortage is observed throughout the country during peak period of different agricultural operations and thus, escalating agricultural wages year after year. We do not have cost effective small size machines for different operations in rice farming.



State	Cost of cultivation (C <sub>2</sub> )* (Rs. ha <sup>-1</sup> )	Gross return (Rs. ha <sup>-1</sup> )	Profit over $C_2 \cos(Rs. ha^{-1})$	Cost of cultivation $(A_2+FL)^{**}$ (Rs. ha <sup>-1</sup> )	Profit over A <sub>2</sub> +FL cost (Rs. ha <sup>-1</sup> )
Andhra Pradesh	78968	83972	5004	51821	32151
Assam	49887	36819	-13068	36000	819
Bihar	40097	43483	3386	26308	17175
Chhattisgarh	45775	45303	-472	30848	14455
Jharkhand	38848	43429	4582	23875	19554
Gujarat	55798	73484	17686	41447	32036
Haryana	78948	119977	41030	45717	74261
Punjab	73254	106826	33572	34041	72785
Himachal Pradesh	37071	46368	9298	26323	20045
Karnataka	68315	81571	13255	48419	33151
Kerala	71972	89927	17955	52905	37022
Madhya Pradesh	41381	37492	-3889	28415	9077
Maharashtra	68262	48758	-19505	54417	-5659
Odisha	56914	47153	-9761	42302	4851
Tamil Nadu	74077	76294	2217	55252	21042
Uttar Pradesh	58982	50417	-8565	39481	10936
Uttarakhand	45634	62115	16481	30396	31719
West Bengal	71840	58956	-12885	54259	4696

### Table 4. State wise costs and returns of rice cultivation (2014-15).

 $C_2$ , operational and fixed costs; \*\* $A_2$ +FL, operational costs including family labour; FL, Family labour.

Source: Ministry of Agriculture, Government of India.

# 6. STRATEGIES FOR ENHANCING PRODUCTIVITY AND PROFITABILITY OF RICE PRODUCTION

Rice research should aim at tapping genetic resources and utilizing them for breeding rice varieties with higher yield potential, better grain and nutritional quality, enhanced input use efficiency and increased tolerance to major biotic and abiotic stresses through conventional and innovative techniques such as marker assisted breeding, development of transgenics, functional genomics, improvement of degree of heterosis, and improvement of photosynthetic efficiency through  $C_4$  mechanism. Identification of potential new donors for abiotic and biotic stresses and unravelling the underlying tolerance mechanisms will also receive due attention. Genomics, proteomics, metabolomics and phenomics tools will be employed for understanding multiple abiotic/biotic stress tolerance mechanism. Redesigning rice plants for improving photosynthesis and plant productivity under multiple abiotic stress environments through transgenics would also be one of the approaches. Research emphasis will be on improving water and nutrient use efficiencies with special emphasis on conservation agriculture, climate-resilient rice and rice-based cropping and farming



systems. Rice physiology and biochemistry under high CO<sub>2</sub>, ozone and temperature would be unravelled for defining climate resilient rice cultivars. Innovative approaches involving nano-technologies will be taken up for efficient use of fertilizers and pesticides. Use of newer molecules for control of diseases and insect pests, including bio-pesticides, and integrated pest management (IPM) are other areas of focus. Host-parasite/pathogen interaction at molecular level including QTL identification to design suitable control strategy will be the approach for resistance breeding.

Management of rice related knowledge, with due attention on extension services and fostering linkage and collaboration with public, private, national and international organizations are other important areas on which the institute will focus. Strategic planning, priority setting and impact assessment of rice research in India with a global perspective will be taken up to consolidate the gain. Capacity building of scientists, farmers and other stakeholders will be given due importance, so as to be globally competitive and ensure food and nutritional security of the country.

The following options are available for increasing farmers' productivity and income in rice-based systems.

### 6.1. Improving productivity and quality

**6.1.1. Providing quality seed and enhancing seed replacement ratio:** Seed is the critical determinant of agricultural production on which depends the performance and efficacy of other inputs. Quality seeds appropriate to different agro-climatic conditions and in sufficient quantity at affordable prices are required to raise productivity. Availability and use of quality seeds is not a onetime affair. Sustained increase in agriculture production and productivity necessarily requires continuous development of new and improved varieties of crops and efficient system of production and supply of seeds to farmers. Despite a huge institutional framework for seed production both in the public and private sector, availability of good quality seeds continues to be a problem for the farmers. As a result, they prefer to rely on farm saved seeds; seed replacement rate continues to remain low for most crops. As is well known, seed replacement rate has a strong positive correlation with the productivity and production of crops.

**6.1.2. Promoting high-yielding varieties and hybrids:** Due to unavailability of the quality seeds of high yielding varieties and high seed cost of the hybrids, farmers are unable to get those seeds and they prefer to grow their local seed materials. The yield potential of local seeds is low and they are susceptible to many pests and diseases. Although many government programmes are in operation for making HYV and hybrids seeds available to the farmers, yet there is ample scope of promoting high-yielding varieties and hybrid. Further, improved market support will encourage the farmers for adopting high yielding varieties and hybrids.

**6.1.3. Growing nutrient rich and aromatic rice:** At present no support price for farmers for high nutrient rich rice such as high protein rice CR Dhan 310 and CR Dhan 311 or any other specialty rice (aromatic non-basmati) is available. Therefore, for popularization of the variety in suitable lands and for increasing the higher commercial



value of these rice, initiatives from institutional and extension machinery as well as modification in policy decision are collectively required. Higher support price for growers and subsidy for mid-day meal rice are required to give benefits both the poor rice-farmers and our underprivileged children in villages of India.

**6.1.4. Increasing cropping intensity in rice-fallow areas:** About 30% (11.7 Mha) of the area under rice production during kharif season in India remains fallow in the subsequent rabi due to number of biotic, abiotic and socioeconomic constraints. Despite of ample opportunities rice fallow systems have been bypassed in the research and developments for a numbers of constraints. Major rice fallow area (82%) is concentrated on eastern parts of the country. States with larger area of rice-fallows are Chhattisgarh, Madhya Pradesh, Jharkhand, Bihar, West Bengal and Orissa the remaining 18% area lies in the states like Tamil Nadu, Karnataka and Andhra Pradesh and there exists a large scope for expansion of area under pulse crops. Short duration pulses are ideal candidates for their cultivation in such areas. To exploit these rice fallow areas with pulses, location specific and economically viable technology for better performances of pulses are required to be standardize through proper understanding of the system ecology and constraints study.

### 6.2. Increasing input use efficiency

**6.2.1. Crop planning:** Current land use pattern for agriculture in many states are not based on principles of comparative advantage. Crops pattern in various region are inefficient in terms of resource use and unsustainable from natural resource use point of view. This is resulting into serious misallocation of resources, efficiency loss, indiscriminate use of land and water resources, and adversely affecting long term production prospects. Due to lack of proper crop planning, problems of soil and water degradation are aggravating. So, the need is there for proper crop planning in the country so that it is consistent with natural endowment and resources use efficiency.

**6.2.2. Promoting water harvesting and micro-irrigation:** In many farming areas, readily available water is in short supply. Although the total annual rainfall in an area may be enough to sustain farm needs, it is often distributed very unevenly so that long dry periods are interspersed with periods of intense rainfall. In many cases, a crop is unable to use a high proportion of this water, as much of it is lost through run off or leaching. This may also cause soil erosion and loss of soil nutrients. Hence, there is need to promoting water harvesting and micro-irrigation to achieve per drop more crop. Further adoption of water saving technologies such as direct seeding of rice and system of rice cultivation can save water. For surface-irrigated areas, a properly leveled surface with the required inclination according to the irrigation method is absolutely essential. Traditional farmers' methods for leveling by eyesight, particularly on larger plots, are not accurate enough and lead to extended irrigation times, unnecessary water consumption, and inefficient water use. With laser leveling, the unevenness of the field is reduced resulting in better water application and distribution efficiency, and improved water productivity.



**6.2.3. Using soil health card and site-specific nutrient management:** The soil health card carries crop wise recommendations of nutrients/fertilizers required for farms, making it possible for farmers to improve productivity by using appropriate inputs. Under current management practices, nutrient use efficiency are low and farmers often fail to apply nitrogen (N), phosphorous (P) and potash (K) in the optimal ratio to meet the need of crops. Site Specific Nutrient Management (SSNM) provides an approach for feeding crops with nutrients as and when needed. The SSNM eliminates wastage of fertilizer by preventing excessive rates of fertilizer and by avoiding fertilizer application when the crop does not require nutrient inputs.

**6.2.4. Promoting farm mechanization and solar energy:** Intensification of mechanization is one of the most important factor for increasing agricultural activities and production as well. Productivity of farms depends greatly on the availability and judicious use of farm power. Agricultural implements and machines enable farmers to employ the power judiciously for production purposes. Agricultural machines increase productivity of land and labour by meeting timeliness in farm operations. Mechanization has the advantages of proper utilization of resources, reducing drudgery in farm operations, timely execution of various agricultural operations and best use of the available soil moisture. Switching over from animal power to mechanical and electrical power for enhanced power availability for various farm operations, reduce cost of operation, and crop diversification. Promoting use of renewable energy in farm equipment segment such as solar-powered pumps may have the immense potential in farm operations and can create alternate source of revenue for the farmer by selling the additional power.

### 6.3. Reducing crop loss

**6.3.1. Adopting plant protection measures:** Plant protection continues to play a significant role in achieving targets of crop production. The major thrust areas of plant protection are promotion of integrated pest management (IPM), ensuring availability of safe and quality pesticides for sustaining crop production from the ravages of pests and diseases, streamlining the quarantine measures for accelerating the introduction of new high yielding crop varities, besides eliminating the chances of entry of exotic pests.

**6.3.2. Promoting resistant varieties and e-surveillance:** The crop losses due to pests and diseases occur despite increased pesticide use, which highlight the need to develop sustainable approaches for pest control with less reliance on chemical inputs. To address concerns regarding human health, environmental safety and pesticide resistance, plant defensive traits could be exploited more widely in crop protection strategies. Further, it is essential to have a pest monitoring system, which will check the spread of disease pest and the crop loss.

**6.3.3. Crop insurance to mitigate risks at affordable cost:** Crop insurance provides required coverage to farmers against production loss for crops. It also offers preventive planting and repellant security. A crop insurance plan could prove a life-saver by providing compensation. Therefore, farmers should be advised to take up insurance plans to compensate their income during adverse years.



**6.3.4. Weather services and forecasting system:** Every year crops are damaged by pest and diseases. Due to lack of proper operational forecasting system for the incidences of pests and diseases, it becomes difficult to adopt plant protection measures at right time. It has been established with fair degree of accuracy that climate/weather play major role in the incidences of pests and diseases. Thus, there is great scope of utilizing meteorological parameters for the advance information of the occurrences of pests and diseases and ultimately scheduling of prophylactic measures can be taken scientifically and judiciously.

### 6.4. Diversification of rice areas with low productivity

**6.4.1. Dairy husbandry for small farmers:** Dairying is an important source of subsidiary income to small/marginal farmers and agricultural labourers. The manure from animals provides a good source of organic matter for improving soil fertility and crop yields. The cow dung gas from the dung is used as fuel for domestic purposes as also for running engines for drawing water from well. The surplus fodder and agricultural by-products are gainfully utilized for feeding the animals. A large portion of draught power for farm operations and transportation is supplied by bullocks. Since agriculture is mostly seasonal, there is a possibility of finding employment throughout the year for many persons through dairy farming. Thus, dairy also provides employment throughout the year. The main beneficiaries of dairy programmes are small/marginal farmers and landless labourers.

**6.4.2. Promotion of intensive vegetable and fruit production:** By following intensive vegetable production, and planting fruit trees, farmers can get maximum profit from the farm. Good planning and attention to the production and marketing practices can help the farmers to attain high level of profit.

**6.4.3. Promotion of ancillary activities like poultry, beekeeping and fisheries:** Small and marginal holdings account for about three-fourth of the total operational holdings in the country, operating over one-fourth of the total area. Majority of small and marginal farmers cultivate mainly low value, subsistence crops. In the absence of adequate farm and non-farm employment opportunities, they are also forced to live below poverty line. The situation is likely to worsen because of the growing pressure of population on land and the limited scope of increasing additional production through subsistence farming. Hence arises the need for commercialization and diversification of small farms within and outside agriculture and their proper integration with local and global markets. This is intended not only to liberate the small and marginal farmers from the poverty trap, but also to meet the country's growing demands for meat, fish and eggs.

**6.4.4. Strengthening organic food program:** Organic food is preferred as it battles pests and weeds in a non-toxic manner, involves less input costs for cultivation and preserves the ecological balance while promoting biological diversity and protection of the environment. Generally, organically grown food fetches better price in the market.



## 6.5. Creation of Infrastructure, market price realization and value addition

**6.5.1. Infrastructure:** The availability of storage facilities is not sufficient for storage during peak harvesting period. Similarly, the availability of number of regulated markets is not sufficient to cater to the needs of farmers. As a result, the MSP is not effective in eastern states. Government should review the state of procurement operations in the six eastern states, *viz*. Assam, Bihar, Jharkhand, Odisha, Uttar Pradesh and West Bengal, where the support price mechanism is not effective, on priority for taking improvement measures.

**6.5.2. Community/co-operative farming with crop-value chain:** Institutional reforms are necessary to generate collective actions through co-operative avenues to overcome the development deadlock created due to small and uneconomical holding sizes. This is essential not only to enhance collective bargaining power of the farmers but also to inculcate the spirit of submerging the personal interests in collective welfare. Earlier system of co-operative farming, emerging group approaches such as the self help groups (SHGs) and prospects of creating farmers' corporations need to be explored thoroughly.

**6.5.3. Using crop biomass to make products through small industry:** Biomass pellets can be sold commercially as the main fuel for industrial boilers and replace coal. Micro-pelletization should be incentivized and its local usage promoted. There are other small-scale industries such as cardboard manufacturing and mattress production that can utilize crop residues. Straw can also be used for substrata for mushroom cultivation (Pathak et al. 2012).

**6.5.4. Creation of a national e-market:** National farm market allows farmers and traders to sell their produce to buyers anywhere in the country. National farm market addresses the modern day market challenges by creating a unified market through online trading platform, both, at state and national level and promotes uniformity, streamlining of procedures across the integrated markets, removes information asymmetry between buyers and sellers and promotes real time price discovery, based on actual demand and supply, promotes transparency in auction process, and access to a nationwide market for the farmer, with prices commensurate with quality of his produce and online payment and availability of better quality produce and at more reasonable prices to the consumer.

**6.5.5. Agribusiness Incubation Centres to promote agri-preneurship:** The Agri-Business Incubation (ABI) program aim to promote entrepreneurs in public-private partnership mode that maximizes the success quotient of start-up entrepreneurs by offering them best opportunities with minimum risk. Effective communication, coordination and cooperation among the various nodal centres, umbrella consortium and the industry are inevitable for the successful implementation of the schemes.



### 7. CONCLUSIONS

In order to meet future rice demand, increasing production and productivity of rice is essential. This has to be done in the face of growing shortage of land, labour and water for rice farming. As the profit margin in rice cultivation has decreased, there is need to not only increase in yield per se but also bring efficiency in input use in rice production. The goal of rice research should be in developing profitable and resilient rainfed rice farming system with a vision of enhancing productivity, profitability and resilience for ever-green rice farming with high-quality research, partnership and leadership in rice science. The thrust areas research should include (1) genetic enhancement for improving productivity, quality and climate resilience of rice; (2) ecosystem management for higher input-efficiency and lower environmental footprints; (3) value-addition with improved quality, co-farming, processing and marketing and (4) accelerating technology delivery, capacity building and policy formulation. The rice-research in the past has made immense contributions in developing and demonstrating technologies for improved rice farming. It, however, needs to be strengthened to address the emerging challenges of low productivity and low income of rice farmers in the face of environmental changes. A multi-disciplinary and participatory research should be adopted to address the emerging challenges and make rice farming more productive, profitable and climate resilient.

#### References

- Kumar P, Joshi PK and Birthal PS (2009) Demand projections for Foodgrains in India. Agricultural Economics Research Review 22:237-243.
- Pathak H (2015) Greenhouse gas emission from Indian agriculture: Trends, drivers and mitigation strategies. Proceeding of Indian National Science Academy 81(5): 1133-1149.
- Pathak H and Ladha JK (2006) Rice: Environmental issues. Indian Farming 56(7): 46-49.
- Pathak H, Bhatia A and Jain N (2012) Crop residues management with conservation agriculture: Potential, constraints and policy needs. Indian Agricultural Research Institute, New Delhi, vii + 32 p.
- Samal P (2009) Future technological needs for rice crop in India: Socio-economic concerns. In 'Forecasting future technological needs for rice in India', Indian Agricultural Statistics Research Institute, New Delhi, pp. 14-24.
- Samal P (2013) Growth in production, productivity, Costs and profitability of rice in India during 1980-2010. In P Shetty, MR Hedge and M Mahadevappa (Eds.) Innovations in Rice production, National Institute of Advanced Studies, Bangalore 12, pp. 35-51.
- Tilman D, Cassman KG, Matson PA, Naylor R and Polasky S (2002) Agricultural sustainability and intensive prodution practices. Nature 418: 671-677.
- Wassmann R, Jagadish SVK, Heuer S, Ismail A, Redona E, Serraj R, Singh RK, Howell G, Pathak H, Sumfleth K (2009) Climate change affecting rice production: The physiological and agronomic basis for possible adaptation strategies. Advances in Agronomy 101: 59-122.
- Wassmann R, Jagadish SVK, Sumfleth K, Pathak H, Howell G, Ismail A, Serraj R, Redona E, Singh RK, Heuer S (2009) Regional vulnerability of climate change impacts on Asian rice production and scope for adaptation. Advances in Agronomy 102: 91-133.\*



### **Rice Genetic Resources: Collection, Conservation, Maintenance and Utilization**

## BC Patra, BC Marndi, P Sanghamitra, S Samantaray, N Umakanta and JL Katara

### SUMMARY

The increased demand for rice will have to be met from less land, less water, less labour and fewer chemicals under changing climate. Can we meet the challenges to rice productivity, stability and nutritional quality improvement by strategic use of the available germplasm resources? Can India, having more than 106,000 germplasm accessions in the National Gene Bank effectively make use of these huge resources? Past experience suggests that germplasm still holds the key to our food and nutritional security of future generation. It is well known that the traditional rice varieties and their wild relatives constitute an invaluable gene pool in terms of resistance/tolerance to biotic and abiotic stresses, which can be exploited for developing modern new generation rice varieties having enough resilience to sustain adverse climatic changes. All these issues have been dealt in this chapter under the ongoing Institute multi-disciplinary research project.

### **1. INTRODUCTION**

The plant genetic resources (PGR) constitute the basic raw material for any crop improvement programme. It may consist of seed or vegetative propagules (tuber, sucker, rhizome, cutting, seedling etc.) of plants, which contains the functional units of heredity. They are generally referred to as germplasm or genetic resource material. In fact, Sir Otto Frankel coined the word 'Genetic Resources'. Rice (Oryza sativa L.) is one of the most important cereal food crops for more than one-half of the world population and provides 50-80% of daily calorie intake. Rice is grown in more than 115 countries. In India, it is cultivated under a wide range of growing conditions, such as below sea level farming in Kuttanad in Kerala to high altitude farming in the Himalayas. Because of its adaptation to such variable agro-ecosystems, fortunately a rich genetic diversity and variability is encountered which helps sustain the adverse alterations in temperature, precipitation as a result of climate change. There are varieties, which can withstand submergence during flood and there are others which can grow under moisture stress during drought condition and also at soil and water salinity. Therefore, it becomes imperative to conserve them for posterity. The search for superior genotypes regarding yielding ability, disease and pest resistance, abiotic stress tolerance or better nutritional quality is very hard, competitive and expensive. Evidently, there is a gap between available genetic resources and breeding activities. However, with the advent of modern genomic tools the scope for use of vast genetic resources has increased. Newer strategies must be designed, first for an elaborate evaluation, and subsequently for efficient utilization of the diverse germplasm resource



so painstakingly collected and conserved in the gene banks. Accelerated genetic gains in rice improvement are needed to mitigate the effects of climate change and loss of arable land, as well as to ensure a stable global food supply. The enormous rice genetic diversity available in the gene banks will be the foundation of the genetic improvement of the crop through unraveling the new genes and traits that will help rice producing farmers who are facing the challenges brought about by climate change, pests and diseases, and other unfavourable conditions.

### 2. ORIGIN AND EVOLUTION OF CULTIVATED RICE

Rice is cultivated as far north (53°N) on the border between Russia and China, and as far south as central Argentina (40°S). It is grown in cool climates in the mountains of Nepal and India, and under irrigation in the hot deserts of Iran and Egypt. It is an upland crop in parts of Asia, Africa and Latin America. At the other environmental extremes are floating rice, which thrive in seasonally deeply flooded areas such as river deltas - the Mekong in Vietnam, the Irrawady in Myanmar and the Ganges-Brahmaputra in eastern India and Bangladesh.

The centre of origin and centers of diversity of two cultivated rice species i.e. *Oryza sativa* and *O. glaberrima* have been identified using genetic diversity, historical and archaeological evidences and geographical distribution. It is generally agreed that river valleys of Yangtze, Mekong Rivers could be the primary centre of origin of *Oryza sativa*. The foothills of the Himalayas, Chhattisgarh, Jeypore tract of Odisha, north eastern India, northern parts of Myanmar and Thailand, Yunnan Province of China are some of the secondary centers of diversity for Asian cultigens. There are 22 agro-biodiversity hotspots in India, out of which five hotspots fall in eastern region of the country, of which the Koraput region covering part of northern Eastern Ghats is of great concern as the upland short duration drought avoiding *aus* types have been originated here.

The Inner delta of Niger River and some areas around Guinean coast of the Africa are considered to be the centre of diversity for the African cultivated species of *O. glaberrima* (Chang 1976; Oka 1988). It is also assumed that the Asian annual wild species *O. nivara* has given rise to the Asian cultivated species *O. sativa* and the African annual wild species *O. barthii* to the African cultivated species *O. glaberrima*.

The diversity and variability within the Asian cultivated rice (*O. sativa*) is enormous. Some controversy exists over when and where rice was domesticated. It is fairly safe to say that rice was being cultivated at least 10,000 years ago and that it was domesticated from its wild ancestor *O. rufipogon* (Khush 1997). Two major sub groups of rice, *indica* and *japonica*, led rice genetic resources specialists to conclude that there were two centers of origin. One was thought to be in the tropical regions of South Asia where *indica* rice varieties dominated and the other near Central China where *japonica* rice dominated (Londo et al. 2006). It has generally been recognized that genetically the *japonica* (*sensu stricto*) is a fairly homogeneous group whereas the *indica* is highly heterogeneous group (Jennings 1966).



### 3. WILD RICE

Genus *Oryza* consists of 24 species, of which two are cultivated and rests are found wild in different parts of the world; all of them grow in the tropics only. There are two species that are close relatives of the cultivated rice and are believed to be the progenitor species of the cultivated ones. One of these, *Oryza rufipogon*, grows wild in India, China, Southeast Asia and South Asia. The other one, known as *O. barthii* (syn. *O. breviligulata*) grows wild in northern part of tropical Africa. These two wild species of rice attracted the attention of Neolithic man for their grains for his subsistence especially when he had not enough wild animals for hunting or wild fruits to gather. In fact, some of the aborigines still continue to collect seeds of wild rice in India, Southeast Asia and Africa. Recent study at this institute on morphological and molecular aspect confirms the validity of two wild rice species of same AA genome and there is distinct speciation between perennial *O. rufipogon* and annual *O. nivara* populations (Samal et al. 2018).

### 4. WEEDY RICE: MYSTERY IN EVOLUTION

Weedy rice appears as hybrid swarms due to introgression of genes between wild and cultivated species in nature. In Asian rice, it is known as Oryza spontanea whereas in African context it is said as O. stapfii. It grows faster; produces more tillers, panicles and biomass; makes better use of available N; shatters earlier; has better resistance to adverse conditions; and possesses longer dormancy in soil. Because of its high competitive ability, it becomes a serious threat to rice growers worldwide. It is also called as red rice because of its red pericarp. One of the major constraints/bottlenecks to attain the potential yield target of the present improved rice cultivars is considered due to the contamination of weedy rice in the cultivated areas. Due to conspecific form of cultivated rice makes similarity of morphological and physiological appearance between weedy rice and cultivated rice in the field, it is very difficult to discriminate the weedy rice phenotypically from the cultivar/variety of rice at the vegetative stages. Therefore, chemical control measures to manage weedy rice in conventional rice cultivars are not advisable. Some weedy rice also have inherited traits linked to wild rice such as red pericarp, black hull, long awn, light seed weight, strong seed dormancy and easy seed shattering which leads to loss of grain during harvesting in the field. There is also very high level of genetic variability and plasticity found within and among weed populations (Green et al. 2001) as the growth of weedy rice varies considerably among different biotypes due to differences in plant height, tillering, or leaf-producing capacity. Since, evolution of the weedy rice is not completely understood, a preliminary study was conducted to evaluate the genetic diversity of weedy rice lines found in the state of Odisha which includes seventy five weedy rice collected from different locations of Odisha, India of which fifteen wild rice (six accessions of Orvza rufipogon, nine accessions of Orvza nivara), six cultivated rice including three each of landraces and high yielding cultivars (Ngangkham et al. 2016).

A set of SSR molecular markers from different chromosomes were used for diversity analysis in 96 weedy rice and found to be robust enough to be used for diversity analysis. The observed heterozygosity (Ho) in analysis was found low which might be due to autogamous nature of rice crop (Nachimuthu et al. 2015) and less segregation or selection of stable progenies in the present samples. The genetic diversity was found to be higher than the other weedy rice populations studied. The genetic similarity coefficient of the whole 96 samples varied from 0.60 to 1 and distributed in different clusters. This result suggests a complex and unclear evolutionary process of weedy rice in Odisha, India. Thus, the considerable higher level of genetic diversity in weedy rice lines of Odisha indicates a complicated type of origin of weedy rice in this region of India which might be due to either adoption of direct seeded rice growing technique in this region or reduced weed control practices owing to limited human labour input. The genetic similarity coefficient of the whole 96 samples varied from 0.60 to 1 and diversifying the whole weedy rice lines into distinct groups after their evolution. Thus, the present investigation revealed that the origin of some of the weedy rice of Odisha is probably through the hybridization between the wild rice and rice cultivars cultivated in the nearby areas by the farmers.

Recent changes in farming practices and cultivation methods along with less weed management may have promoted the re-emergence and divergence of weedy rice. The abundant genetic diversity of weedy rice populations accompanied by the changes of farming practices may complicate weedy rice control in future and consequently threaten rice production. Thus, effective methodologies for weed control and management must be developed to prevent weedy rice from extensive spreading and infestation.

### **5. NERICA RICE**

West Africans domesticated *Oryza glaberrima* about 3,500 years ago. The Asian species *O. sativa* reached Africa about 450-600 years ago and slowly displaced the native rice because of low harvest. By the 1990s, native African rice was reduced to a few pockets on scattered farms. Then Sierra Leonean plant breeder Monty Jones and his colleagues found a way to create a fertile hybrid between African and Asian rice. Called "*Nerica*" (New Rice for Africa), it could yield a bumper harvest like its Asian parent, but it was as tough as its African side, resistant to drought, pests and diseases. Scientists have bred many varieties of *Nerica* and farmers have started growing them. This new rice, descended from an endangered species, is helping Africa to feed itself, yet this opportunity would have been lost if *O. glaberrima* had gone extinct.

# 6. EXPLORATION AND COLLECTION OF RICE GERMPLASM

In the past, the scientists involved with crop improvement programme at different research stations undertook the evaluation of germplasm and identified several donors. They were utilized for crop improvement program and 394 varieties were recommended for general cultivation, as pure line selections. In 1955, when Dr. N. Parthasarathy was Director, the NRRI undertook its first planned exploration and collection mission of rice germplasm in the erstwhile Jeypore tract (now Koraput district of Odisha). The collection programme continued for five years (1955-59) by a team of scientists led by



Dr. S. Govindaswami and supported by a scheme sanctioned by the ICAR. This mission was popularly known as Jeypore Botanical Survey (JBS) and was the first of its kind, ever organized in the world to collect rice germplasm (Chang 1989). The team explored about 27,000 km<sup>2</sup> and collected a total of 1,745 cultivated rice and 150 wild rice accessions (Govindaswami and Krishnamurty 1959). Recently in 2010, FAO recognized Koraput region as one of the Globally Important Agriculture Heritage Systems (GIAHS). Later when Dr. R.H. Richharia became Director of NRRI, he introduced 67 varieties from Taiwan, tested them, two or three cultures were dwarf types and one of them was identified as Taichung Native 1 (TN 1) which laid the foundation for Green Revolution in the country. Simultaneously, a PL-480 project on collection of rice germplasm was operative in North east during 1967-72 with Dr. M.S. Swaminathan and Dr. S.V.S. Shastry at IARI, New Delhi and was popularly known as Assam Rice Collection (ARC). During 1970-79, a special programme was undertaken to collect rice germplasm from all the rice growing districts of Madhya Pradesh by Dr. R.H. Richharia after he left NRRI in 1969. He explored 42 districts and collected a total of 19,226 accessions which formed the Raipur Collection. A special drive for upland paddy varieties under cultivation in Andhra Pradesh, Karnataka, Maharashtra, Madhya Pradesh, Uttar Pradesh, Odisha and West Bengal further resulted in collection of 1,938 cultivars. In 1975, a comprehensive exploration and collection programme was drawn for the whole country especially for the traditional rice growing areas of Karnataka, Maharashtra, Madhya Pradesh, Uttar Pradesh, Bihar, West Bengal and Odisha covering 30 districts of 7 states. This programme was popularly known as National Collection from States (NCS) and resulted in collection of 1,038 accessions. Increased interest in herbal medicines during last few decades has necessitated collection of rice germplasm with special emphasis on their medicinal value from Bastar region of Chhattisgarh and Kerala for the world famous 'njavara' rice. During recent evaluation, few landraces/farmers' varieties from Assam have been found to have high level of protein (14-15%). Traditional landraces like Bindli from Uttar Pradesh is now reported to have high Zn (>50 ppm) in brown rice apart from having strong aroma. Studies on the evolutionary changes in the traditional varieties grown in particular regions have been started to find out the reasons of disappearance/extinction of primitive varieties/landraces for the farmers' field (Chourasia et al. 2017).

### 7. COLLECTION OF TRAIT SPECIFIC GERMPLASM

### 7.1. Medicinal rice

The documentation of indigenous traditional knowledge on the medicinal and nutritional significance of red rice is another aspect which is gaining momentum due to recognition of *njavara* rice of Kerala as one of the regions of Geographical Indication (GI) of Goods Act 1999 under the Intellectual Property Right. This was the first time that a rice variety of Kerala received GI Registry in 2008. Studies found that *njavara* has increased level of protein and amino acid in the organically grown seeds; thus it should be developed as baby food and a health product to save this wonder rice from extinction. Seventy two accessions of rice germplasm were collected from Bastar region of Chhattisgarh in which some medicinal rice namely Gudmatia, Bhejari, Danwar, Baisur, Gathuwan were also reported.



### 7.2. Saline tolerant rice

Fifty one accessions of saline tolerant rice mostly from Pokkali region of Kerala (one of the potential regions for GI) were collected, characterized and evaluated for better utilization.

### 7.3. Basmati rice

Eighty eight accessions of long slender basmati rice germplasm were collected from eight districts of western Uttar Pradesh and six districts of Haryana state in collaboration with NBPGR during early 1990s.

### 7.4. Aromatic short grained rice

Sixty seven accessions of short grained scented rice 'Kalanamak' germplasm were collected from eastern Uttar Pradesh, which has been evaluated.

### 7.5. Boro rice

A total of 208 accessions of Boro rice germplasm were collected from Assam, north Bihar, north Bengal and eastern Uttar Pradesh during early 2000.

### 7.6. Bao rice

About 126 accessions of Bao rice germplasm were collected from deep water areas of Assam and Meghalaya, and evaluated for utilization in breeding programme.

### 7.7. Aman rice

A set of 69 accessions of Aman rice germplasm were collected from West Bengal.

### 7.8. Cold tolerant rice

A set of 116 accessions of cold tolerant rice germplasm were collected from hilly regions of Arunachal Pradesh.

### 7.9. Wild and weedy rice

About 495 accessions of wild rice germplasm (*O. nivara, O. rufipogon*, and *O. coarctata (Syn: Porteresia coarctata)* were collected in 12 exploration trips from Odisha and West Bengal under National Agricultural Technology Project (NATP). Apart from this about 223 accessions of weedy rice (*O. sativa f. spontanea*) have also been added to the gene pool.

### 7.10. Specialty rice

Attempts are on to collect aromatic rice, soft rice, wine rice, glutinous or waxy rice, colour rice (brown, green, black, red), beaten rice, pop rice, organic rice, nutritional rice etc.

### 8. GERMPLASM INTRODUCTION

When the International Rice Commission (IRC) recognized NRRI as a centre for the maintenance of world genetic stocks of rice, many varieties of south and Southeast



Asian countries were introduced to the country for their maintenance at NRRI, this provided an opportunity to Indian scientists to test and recommend few of them for general cultivation in the country. Since its inception in 1946 till 1977, Director, NRRI continued to remain in charge of overall supervision of the world genetic stock for multiplication and maintenance of the FAO designated germplasm being run at 5 countries i.e. India, Indonesia, Japan, Pakistan and USA. The world genetic stock was comprising of japonicas, indicas, bulus and floating types. Again, when NRRI was recognized as the main centre for the inter-racial hybridization programme between japonicas and indicas during 1950-1964, many exotic japonica rice germplasm were introduced into India. The participants of the southeast and south Asian countries came with their own rice varieties for hybridization. This further provided opportunity to Indian rice scientists to study the varieties of other countries. Some of the japonicas when tried in temperate hilly regions were found suitable for direct introduction. Many *japonica* varieties (Aikoku, Asahi, Fukoku, Gimbozu, Norin 1, Norin 6, Norin 8, Norin 17, Norin 18, Norin 20, Rikuu 132, Taichu 65) were crossed with the popular varieties of Odisha (T 90, T 812, T 1145, BAM 9) and the progenies were grown at the three Rice Research Stations (Bhubaneswar, Berhampur and Jeypore) for further selections. In all, 192 improved local varieties were selected and a total of 710 different indica x japonica crosses were made. The F, seeds were distributed to many countries for further crop improvement programme. Only four varieties were released; Malinja and Mahsuri released in Malaysia, ADT 27 in Tamil Nadu state of India and Circna in Australia.

# 9. GERMPLASM CHARACTERIZATION, DOCUMENTATION AND SEED SUPPLY

Characterization is the description of plant germplasm, which involves determining the expression of highly heritable characters ranging from morphological or agronomical features to seed proteins or molecular markers. It results in better insight in the composition of the collection and the coverage of genetic diversity. Every year new germplasm accessions are collected and conserved in Gene bank of NRRI which is characterized for utilization in the breeding programme. So information on germplasm also helps to facilitate the exchange of materials and information among gene banks and help the users in experimenting with conserved germplasm.

### **10. STATUS OF RESEARCH**

At National Rice Research Institute, all the germplasm collections including wild and weedy rice are characterized at appropriate stages of plant growth and maturity for agro-morphological traits based on the descriptors which include 19 qualitative and 11 quantitative characters. These materials after characterization are harvested, processed, packed and sent to National Gene Bank for long term storage (LTS) and also a set of it is conserved at ICAR-NRRI under medium term storage module. The details of germplasm characterized at NRRI during last five years are given in Table1.



Year	No. of accessions characterized	Type of germplasm
2012-13	2545	Released varieties and land races of India
2013-14	600	Landraces of India
2014-15	6406	Landraces, wild and weedy rice of India
2015-16	5800	Landraces, wild and weedy rice of India
2016-17	5800	Landraces, wild and weedy rice of India

Table 1. No. of germ	plasm characterized	l during last five	vears (2012-17).

With the aim of creating database, 14000 accessions of morphologically characterized rice germplasm were documented (Tables 2 & 3). The data revealed that majority of the accessions were with green basal leaf sheath, green leaf blade, well exserted panicle, intermediate type, long fully awned, intermediate threshing, white kernel colour, some aromatic, erect flag leaf and fast leaf senescence type (Fig. 1).

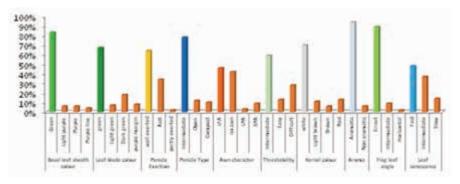


Fig. 1. Variations observed in qualitative characters of 14000 rice germplasm accessions

	Possible donor for resistance			
Diseases	Landraces	Improved Varieties		
Blast	Tetep, Tadukan, Gampai, Peta,	ADT 29, ADT 25, CO 43,		
(Pyricularia	Sigadis, Dular, Kalamdani, Tarabali,	MTU 3626, MTU 6203,		
oryzae)	Mugisali, Madrisali, Sollpona,	MTU 7014, MTU 9992,		
	Rongaahu, Manoharsali, Andrewsali,	WGL 26889, WGL 47969,		
	Gajepsali, Beganbisi 2, Rongagutia,	WGL 47970, MTU 993,		
	Kolimekuri, Rikhojoi 2, ARC 7098,	BJ-1, NLR 145, PTB 10,		
	AC-55 (CH-55), AC-8368 (BJ-1),	NLR 36, CO 4, CO 25, CO 29,		
	AC-8369 (S-67), SM-6, SM-8, SM-9,	CO-30, MTU 9993, Saleem,		
	CP-6, AC-293 (AKP-8), AC-294	Thikkana, Kotha Molagoli,		
	(AKP-9), AC-360 (PTB-10),	Kulu-72, Pinakini, Swarna muli		
	AC-26904 (Tetep), Fukunishike CO-4,	Dular, Vajram, Prahlad, Lacrose-		
	CO-29, Tadukan, Zenith, Carreon	Zenith-Nira, BJ1, Karjat,		

Table 2. Important donors in	lentified against major diseases.
------------------------------	-----------------------------------

Contd.....



	Possible donor for resistance			
Diseases	Landraces	Improved Varieties		
Bacterial leaf blight ( <i>Xanthomonas</i> oryzae)	Dholamula, Moinasail, Kartik kolma, Japorisali, Gajepsali, Jatiosali, Kola ahu, Ahusuri, ARC 5827, AC-33523 (Tarical), AC-33557 (Dulla karma), AC-33562 (Kangpui), AC-3094 (TKM-6), AC-8368 (BJ)-1, AC-26903 (DV-85), Chinsurah boro-II, Somera mangga, Wase Aikoku 3, Malagkit, Sung son	Pallavi, TKM 6, Sigadis, Pelita 1, MTU 15, MTU 16, ASD 5, T 1069, ARC 18562, MTU 4870, MTU 2400, MTU 3626, MTU 6203, MTU 7014, MTU 9992, Swarna, PLA 9180, BPT 3291, Mahsuri, RNR 10786, PNR 2736, RNR 4970, RNR 10208, AS 330.		
Rice Tungro Virus ( <i>Nephotettix</i> malayanus)	Kataribhog, Latisail, Sigadis, Ambemohar, Habiganj, ARC 14766	BJ 1, PTB 18, W 1263, Gampai 15, Pankhari 203, ARC-7125, ARC-7149, DW-8, AC-368 (PTB-18), AC-5079 (Kataribhog), Bhagirathi, Boitalpakhia, AC-34558 (Nalini), AC- 17933 (Kamod- 153), AC- 34650 (Usha), AC-273 (ADT-20), AC- 297 (ASD-1), AC-304 (CO-1), AC-315 (CO-12), AC-351 (PTB-1), AC-360 (PTB-10) AC-3094 (TKM-6), AC-8396 (CB-1)		
Helmintho sporium oryzae	Bhatta Dhan.	Ch 13, Ch 45, BAM 10, AC 2550, ADT 29, CO 29.		
False smut (Ustilaginoidea virens)	Sugandha, Sabari, Karna, Deepa, Sona, ARC 5378, AC-26570 (ADT-33), AC- 40119 (PTB-23), AC-40124 (PTB-26), AC- 3070 (PTB-32)	Udaya, CO 9, IR 62, MNP 85, BR 16, IR 24, IR 29.		
Stem rot (Helminthospor sigmoideum)	 ium	Basmati 370, Bara 62		
Ragged stunt virus		PTB 21, PTB 33		
Grassy stunt virus		Oryza nivara		
Sheath rot ( <i>Sarocladium</i> oryzae)	Bhatta Dhan	AC- 26904 (Tetep), Ram Tulasi		
Brown Spot	Katak tara, Bhut muri, BAM 10, SR-26B, CH-45, CO-20			



	Donors	
Insect	Land races	Improved Varieties
Brown Plant Hopper (BPH) ( <i>Nilaparvata</i> <i>lugens</i> )	Dhoiya Bankoi, Sahiba, Salkathi, ARC-6650 (Gomiri bora), AC-34969 (Baidya raj), AC-34993(Ghusuri), AC-34997(Jhupjhupa), AC-35014 (Nal dhan), AC-371 (PTB-21), AC-40634 (PTB-33), AC-30300 (MR-1523), AC-35184 (Dhoba numberi), AC-35228 (Jalakanthi), AC-35066 (Banspati), AC-35070 (Panidubi), AC-35108 (China bali), AC-17912 (Ganga sagar), AC-20363 (Kalachudi), Tarapith, Haldi ganthi ARC 7080, ARC 14766, NCS 91, NCS 131, NCS 707, Milyang-55, PTB 43, PTB 21, ARC 6248, ARC 6605, ARC 6619, ARC 5757, ARC 6158, ARC 6102, ARC-6650 (Gomiri bora), AC-34969(Baidya raj), AC-34993(Ghusuri)	Leb Mue Nahng, Udaya, CR 1009, PTB 18, Vajram, Pratibha, Nandi, Chaitanya, Krishna veni
Gall Midge (Orseolia oryzae)	ARC 5984, ARC 10660, ARC 6605, Leuang 152, ARC 5959, ARC 13516, ARC 14787. HR 13, HR 14, Ratnachudi, Eswarakora, HR 12, HR 42, HR 63, AC-35(Ningar small), AC-39 (CNAB white rice), AC-210(Bhadas-79), AC-391(Bikiri sannam), ARC-5984 (Suto syamara), ARC-10660, ARC-12508(Khauji), ARC-12586 (Vale matse), ARC-12588 (Amamma mats ARC-12670(Nien sah), ARC-13166 (Jaks ARC-13210(Yangbelok), ARC-13166 (Jaks ARC-13210(Yangbelok), ARC-14915 (Maich dol), ARC-14967(Galong), AC- 352 (PTB-2), AC- 362 (PTB-12), AC- 371 (PTB-21), PTB-24, AC-26704 (Phalguna) and Leaung-152	Pothana, Phalguna, RP 140, ORS 677, CR157-212, CR157-303, Kakatiya, Divya se),
White Backed Plant Hopper (Sogatella furciferd	ARC 5803, ARC 6064, ARC 7138, ARC 7318, ARC 10340 a)	IET 6288
Stem Borer (Chilo suppressalis)	ARC 6158, ARC 10386, ARC 10443, NCS 266, NCS 336, NCS 464, ARC 5500, W 1263, AC-3094 (TKM-6), AC-392, AC-267 (ADT-14), AC-8396 (CB-1), AC-344 (MTU-15), AC-20006 (JBS-1638), Tepa-1	TKM 6, CB I, CB II, Manoharsali

### Table 3. Important donors identified against major insect pests.



	Donors				
Insect	Land races	Improved Varieties			
Green Leaf Hopper (GLH) (Nephotettix spp.)	ARC 6606	PTB 2, PTB 21, ADT 14, Vijaya, ADR 52			
Leaf Folder (Cnaphalocrocis medinalis)	ARC 1129, Gorsa, Darukasali, AC-33849 (Bundei), AC-35034 (Hari sankar), AC-33831 (Sunakathi), AC-33832 (Surjana), Juli, AC-35338 (Saru chinamali)	PTB 12			
Yellow Stem borer	AC-33515, AC-33526, AC-33538, AC-33563, ARC-5984, AC-30300 (MR-1523) and AC-30349 (Aganni)				
Nematode	AC-26594 (TKM-6), AC-40083 (MTU-17), AC-467 (Lalnakanda-41), Hasma, Bahagia, AC-40509 (Manoharsali Amla, AC-17134 (Sathia), AC-22899 (Anang), AC-23652 (Kalakeri), Kanyakaprashant	),			

#### 10.1. Sharing of germplasm for Rice improvement programme

Supply or distribution of rice germplasm is an important mandate of the institute for the utilization in crop improvement programmes of the country. Germplasm are supplied to various institutes/organizations through proper signing of Material Transfer Agreement (MTA). Total germplasm supplied to various institutes/ organizations during last five years is detailed in Table 4.

Year	Within institute	Outside organizations	Total
2012-13	2261	1929	4190
2013-14	1466	362	1828
2014-15	4821	458	5279
2015-16	5245	237	5482
2016-17	4937	254	5191
2017-18	3075	177	3252
Total	19544	1488	21032

Table 4. No. of rice germplasm accessions distributed within and outside institute.

### 10.2. Germplasm evaluation and utilization

The genetic erosion has been very fast in recent years due to rapid modernization of the society and genetic diversity has been replaced by introduction of few high yielding varieties. Farmers are leaving their own traditional varieties and growing the improved cultures thereby many of the landraces have become extinct. The need for both *in situ* and *ex situ* conservation is now felt as the paddy cultivation in the country is largely affected by extreme natural calamities after rapid climate change,



through an erratic monsoon. Earlier the biggest challenge was flood, but subsequently other factors like salinity after frequent cyclones and sea water surge, temperature rise and drought like situation in many parts of the country have put the challenge before rice researchers to incorporate these genetic factors in the plant.

The importance of genetic resources is widely recognized. Activities in germplasm banks demand qualified researchers in several areas of knowledge. Besides, the conservation of genetic variability for the future, the actual utilization of available accessions is another important goal. The main factors responsible for the low utilization of plant genetic resources are lack of documentation and adequate description of collections, accessions with restricted adaptability, insufficient plant breeders particularly in developing countries and lack of systematic evaluation of the collections. Low seed availability due to inadequate seed regeneration programs is another barrier to their use (Dowswell et al. 1996). Furthermore, breeder-to-breeder exchange materials are very common and constitute a reasonable alternative to extend genetic variability in breeding programs.

Besides biotic stresses, rice crop frequently faces problems of drought, low temperature, submergence, water-logging, salinity/alkalinity etc. These abiotic stress situations cause drastic reduction in yield and thus varieties with in-built resistance to such stresses are desirable. The germplasm having resistance to such stress situations have been identified (Table 5). The All India Coordinated Rice Improvement Project (AICRIP) was launched in the year 1965 and thereafter, more systematic evaluation against major biotic stress situations was undertaken with multi-location field screening followed by greenhouse evaluation.

After repeated screening of thousands of rice germplasm in simulated condition for different abiotic stresses, some landraces were identified as tolerant to complete submergence. They are Khoda, Khadara, Kusuma, Gangasiuli, Atiranga, Ande Karma, Nahng tip, Kalaputia and so on. In some areas crop suffers from floods when it is submerged under water for up to 10 days. Rice cultivars cannot survive such prolonged submergence. Few rice cultivars have been identified which survive submergence up to 80 cm water depth, for 10 to 12 days at early vegetative stage of the crop. Genetic analysis of one such cultivar, FR 13A revealed that tolerance to submergence is controlled by one major gene. Using FR 13A as a donor, improved rice cultivar, Swarna Sub 1 has been developed and released in India which is gaining popularity among the farmers.

Direct seeding is common in rainfed lowlands. In eastern India sometimes early rain causes water stagnation in the field just after sowing which results in poor crop establishment. Two cultivars namely Panikekoa, AC 1631 and T 1471 have been identified as anaerobic seeding tolerant germplasm. The anaerobic seeding establishes the crop under water, reduces cost of cultivation, saves crops from birds and rat damage, reduces weed growth and thereby herbicide application accounting all these towards organic farming. Similarly, several new donors were identified for salinity tolerance at seedling stage and they are Pokkali, Orumundakan, Rahaspanjar, Bhaluki, Kamini, Matchal, Ravana, Gitanjali and Talmugur apart from the most popular variety



	Donors			
Stress	Land races	Improved Varieties		
Drought tolerance	Mahulata, Brahman nakhi, Zingsaingma (AC-9387, MNP-387, IC0593953), Sathchali, Nepalikalam, Kodibudama, NC 487, Dagaranga, Mettamolagolukulu, NC 488, ARC 10372, NC 492, AS 180, Hasakumra, Maibi, Koijapori, Bairing, Ahu joha, ARC 10372, Noga ahu, Soraituni, Lakhi, Pera vanga, Bodat Mayang, Prabhabati, <i>O. nivara</i> (BCPW-30, AC-100476, IC-330611) and <i>O. nivara</i> (SRD 01-17, AC-100374, IC-330470), AC-254, AC-263, AC-304, AC-511, AC-2298, AC-3035, AC-3111, AC-3577, AC-9066, AC-9387, ARC-7063, AC-45 (CH-45), AC-40083 (MTU-17), W-691, AC-37077 (Dhan gora), AC-37127 (Black gora), AC-374440 (Salumpikit), AC- 34256 (Kabiraj Sal), AC-34296 (Bombay murgi), AC-35038 (Godhi akhi), AC-35046 (Nadi tikar), AC-35059, (Phutki bari), AC-35060 (Bhuska), AC- 35143 (Baihunda), AC-35452 (Karama)	CR 143-2-2, N 22, MTU 17, Kalakeri, Janaki, AS 313/11, AS 47, Aditya, Tulsi		
Cold Tolerance	AC 540, Siga, Rajai, CB 1,Dholiboro, Dunghansali, Raja Sanula	Boro 33, IRGC 100081, 10114, 10028, Barkat, Kalinga 2, Tella Hamsa, Satya, Gavinda		
Submergence Tolerance	Bhundi (JRS-9, AC 42091, IC575277), Atirang, Kalaputia, Kusuma, Gangasiuli, Solpona, Sail badal, Dhola badal, Kolasali, Boga bordhan, Rongasali, Khajara, Dhusara, Nali Baunsagaja, FR 13A, FR 43B, Chakia 59, CN 540, S 22, Madhukar, AC-24682 (FR-13A), AC-35741(Telgri), AC-35323(Chaula pakhia), AC-35675(Biesik), AC-36107(SL276), AC-36470(Khoda), Khadara, AC-26670(Janki), AC-40844 (Manasarovar), Sarumuli, AC-40916 (Jalamagna AC-40604 (Jaladhi-1), Kanawar			
Deep water	Nagari bao, Kekoa bao, HBJ 1, Jalamagna, Jaladhi 1, Jaladhi 2			
Coastal Saline/ Alkaline	Cherayi Pokkali (AC 39416, IC413644, NC/03-98), Paloi (AC 42169,JRS-100), Rupsal (AC 42465,PSS-74), Talmugur (AC 43228), SR-26B, AC-8532 (Pokkali), Pateni-2, AC-41360 (Nonabokra), AC-35255 (Rahaspanjar), Canning 7, Ravana	SR 26B, Getu, Dasal, Patnai 23, Pokkali, Hamilton, CSR 10, CSR 13, CSR 18, Vikas, Co 43		
Water-logging	Tilakkachari, NC 496, Kalakher sail	Jhingasail, Patnai 23		

## Table 5. Important donors identified against abiotic stresses.



SR 26B. Drought is another major abiotic stress that adversely affects the crop leading to low productivity. While screening repeatedly over the years, few landraces have been identified as tolerant to vegetative stress drought and they are Mahulata, Sunamani, Naliakhura, Ranganatha Bao, Bhuta, Bibhisal, Brahman Nakhi, Salkain, Gauranga, Karinagin and Kiaketi. Some of such unique germplasm have been registered (Table 6) with NBPGR for special attention and utilization in rice improvement programme across the country.

Sl. No.	Name of germplasm	Year of registration	Registration no.	Important trait
1.	Khoda (PD -27)	2004	INGR No. 04001	Tolerance to complete submergence
2.	T-1471(Kodiyan)	2005	INGR No.05001	Tolerance to anaerobic seeding
3.	Khadara (PD33)	2008	INGR No.08108	Tolerance to complete submergence
4.	Atiranga (RM5/232)	2008	INGR No.08109	Tolerance to complete submergence
5.	Kalaputia (PCP-01)	2008	INGR No.08110	Tolerance to complete submergence
6.	Gangasiuli(PB-265)	2008	INGR No. 08111	Tolerance to complete submergence
7.	Kusuma (PD-75)	2008	INGR No.08113	Tolerance to complete submergence
8.	Mahulata (PB-294)	2008	INGR No.08112	Tolerance to vegetative stage drought
9.	Medinapore (RM5/AK-225; IC-0258990)	2010	INGR No. 10147	Tolerance to complete submergence
10.	Andekarma (JBS-420; IC-0256801)	- 2010	INGR No.10148	Tolerance to complete submergence
11.	Champakali (IC-0258830)-	2010	INGR No.10149	Tolerance to complete submergence

#### Table 6. Registration of Unique Rice Germplasm with ICAR-NBPGR.

Contd.....



Sl. No.		Year of registration	Registration no.	Important trait
12.	Brahman Nakhi (DPS-3)	2010	INGR No.10150	Tolerance to vegetative stage drought stress
13.	Sal kaiin (PB-78;IC-0256590)	2010	INGR No.08112	Tolerance to vegetative stage drought stress
14.	Bhundi (JRS-9; IC0575277; AC42091)-	2014	INGR 14025	Tolerance to complete submergence and having shoot elongation ability
15.	Kalaketki (JRS-4; IC0575273; AC42087)-	2014	INGR 14026	Tolerance to 20 days complete submergence
16.	CR 143-2-2 (IC0513420)	2017	INGR 17019	Tolerance to both vegetative and reproductive stage drought stress
17	Salkathi (AC-35181; PB-289)	2018	INGR17069	Resistance to brown plant hopper (BPH)

#### 10.3. Conservation of Rice Germplasm

Due to the danger of genetic erosion, the effort of developing a cold storage system for rice germplasm was initiated at NRRI in 1984. Meanwhile, during 1986, it was decided to conserve all the germplasm of NRRI at the National Gene Bank. Since then, more than 30,000 rice germplasm accessions have been deposited in the long term storage (LTS) of NBPGR. Under the aegis of the Indo-USAID collaborative project, a cold module was gifted to NRRI. The facility became operative in 1998 with a controlled temperature of  $4\pm2$  °C and  $33\pm5\%$  RH and found to be rather dependable and is meant for medium term storage (MTS) and the seeds are kept viable for 6-8 years. When accessions in the MTS working collection drops below 50 g after seed



supply to indentor or if seed viability falls below 85%, then the accession is rejuvenated. The *japonica* varieties are monitored more frequently than *indica* rice as they have an inherently shorter storage life than *indica* varieties.

The seeds of each of the accessions are dried for reducing the moisture content up to 10-12% and kept in 3-layered aluminum foil pouches for medium term storage. The outer layer of the pouch is polyester of 12 micron; intermediate layer is aluminum of 12 micron and the innermost layer is polythene of 250 gauges. These aluminum foil pouches are stored in cold module at a regulated temperature of 4 °C and 33% relative humidity (RH).

There are about 106,000 accessions of rice germplasm conserved in NGB at -18 °C and with 3-4% RH. After thorough evaluation and screening thousands of germplasm lines over the years, the NRRI has identified several donors resistant/tolerant to different biotic and abiotic stresses as shown in Tables. Utilising these donors in the breeding programme, the Institute has so far released 128 varieties for different rice ecosystems. In the past, NRRI was supplying the germplasm even to the foreign agencies, but in the context of IPR regime, the sharing of germplasm has been restricted to the researchers within the country.

Several categories of germplasm are conserved for different purposes. They are as follows-

- a) Working collection: A collection of germplasm maintained and used by a breeder or other scientist for their own breeding or research, without taking any specific measures to conserve. The collection may have a short life span and the composition of the collection may vary greatly during its lifetime.
- b) Active collection: A collection maintained by a gene bank and used as the source of seeds for active use, including distribution, characterization and regeneration. It is usually conserved under short- or medium-term storage conditions.
- c) Base collection: A collection of seed ideally prepared and held in prescribed conditions for long-term conservation. The seed should be conserved and never used except for
  - i. periodic germination tests
  - ii. regeneration of samples conserved in long-term storage when their viability decreases below threshold
  - iii. regeneration to replace stocks in an active collection after accumulating 3successive generations of regeneration from active collection and
  - iv. as the primary point of rescue when the accession is accidentally lost from all active collections.
- d) Seed file: A small sample of original seed, set aside when a seed sample first arrives at the gene bank, to serve as the definitive reference sample. The seed file should be maintained under dry conditions preventing disease or pest damage, although not necessarily alive. Other seed samples of the same accession, *e.g.* for every new harvest, should be visually cross-checked with the seed file.



e) Safety back-up: Duplicate samples of the base collection, stored in a different gene bank, preferably in a different continent. The storage conditions in the safety back-up should be at least as good as those in the corresponding long-term collection. The holder of the safety back-up has no rights to use or distribute the seed in any way or to monitor seed health or viability. Additional duplication of the base collection to the Svalbard Global Seed Vault provides definitive safety back-up in case of large scale loss of crop diversity. Svalbard Global Seed Vault (SGSV) commissioned at Arctic island of Svalbard near Norway in North Pole in 2008 conserves about 0.8 million germplasm. It is managed by Norway's Department of Agriculture and the Global Crop Diversity Trust (GCDT) under the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) and supported by Bill & Melinda Gates Foundation. Recently, India has deposited 25 accessions of pigeon pea in April, 2014 as the 59<sup>th</sup> Nation.

## **11. KNOWLEDGE GAPS**

Though many rice germplasm are conserved in the gene bank but information on germplasm is not complete. Germplasm without characterization and evaluation data cannot be utilized for crop improvement programme. Hence a systematic characterization, evaluation and documentation of important traits against each germplasm required to be done for its better utilization by the breeder.

## **12. RESEARCH AND DEVELOPMENT NEEDS**

- i. Activities related to genetic resources are characterized by high cost and long term return. Introduction and germplasm exchange, collection, characterization, evaluation, documentation and conservation are essential steps that cannot be overemphasized.
- ii. A database on agro-morphological traits of all germplasm conserved in gene bank need to be prepared.
- iii. Also a National/central rice data-base can be prepared in collaboration with the research centers working in rice along with NBPGR.
- iv. Research work should be oriented towards developing a core collection for better management and utilization of the germplasm. Work in this line has been initiated at this institute (Jambhulkar et al. 2017).
- v. Human resource development by imparting training to persons engaged in PGR activities is required for proper maintenance and conservation of germplasm.

# **13. WAY FORWARD**

i. Germplasm is basic to crop improvement programs for sustainable agriculture. A road map depicting collection sites need to be prepared so that areas which are not covered in the map will be explored and germplasm will be collected and conserved. Future collections should also aim at trait specific collection of germplasm.



- ii. Wild and primitive populations are the reserves of cryptic variability and hence their capacity for adaptive response is high. Such genetic variation is as important as prevalent varietal diversity for genetic conservation. It is, therefore, important to collect and conserve the wild and weedy rice germplasm.
- iii. It has been estimated that even 5% of rice germplasm conserved in different gene banks have not been utilized. Our research should be oriented towards developing a core collection which represents the diversity of entire collection and removes duplicate accessions that will enhance the use of germplasm by identifying diverse source of parents and also will ease in evaluating the germplasm against biotic and abiotic stresses.
- iv. Identifying trait-specific genetically diverse parents i.e., salt tolerance, cold tolerance, drought tolerance, early/late heading, low chilling, tolerance/resistance to particular pests/diseases, adaptability to water logged habitats, tillering capacity, root system, leafiness, etc., apart from quality characteristics are the primary need of the plant breeder for trait enhancement. So identification of new diverse sources will help in better utilization of germplasm in the breeding programmes, aimed at producing agronomically superior cultivars with broad genetic base.
- v. A rice seed file depicting photograph of individual germplasm may be prepared for identification of germplasm and avoiding misrepresentation of germplasm.
- vi. Future works should aim at characterizing the gene bank materials and creating a data base for better utilization in breeding programme. Morphological and molecular characterization of a core/minicore and trait specific subsets will further enhance the usefulness of the germplasm accessions.
- vii. Realizing the importance of genetic diversity, Jeypore tract of Odisha, the Palakkad area of Kerala and Apatani valley of Arunachal Pradesh should be protected as on farm *in situ* conservation sites.

## **14. CONCLUSION**

The importance of genetic resources is widely recognized. Activities related to genetic resources like germplasm introduction, exchange, collection, characterization, evaluation, documentation and conservation are characterized by high cost and long term return. Until recent past, conservation of rice germplasm was synonymous with repeated rejuvenation in the field. This process of maintenance subjected the germplasm to a threat of losing their identity because of random and non-random processes due to sampling. Also loss due to unforeseen natural calamity of the type of super-cyclone and flood devastating the native germplasm cannot be ruled out so far on farm *ex situ* conservation is concerned.

In this chapter we tried to throw light on origin of rice and discussed about wild, weedy and Nerica rice. Various PGR activities like exploration, collection, conservation, characterization, evaluation, documentation, its status at ICAR-NRRI have been elaborately discussed. This chapter emphasizes collection of trait specific germplasm for its utilization in the breeding programme. The development of improved varieties



through introduction and evaluation of germplasm in this institute are also highlighted. Future research work in creating a core/minicore collection and creating data base for better utilization of germplasm are emphasized.

#### References

- Chang TT (1976) The origin, evolution, cultivation, dissemination and diversification of Asian and African rice. Euphytica 25: 435-441.
- Chang TT (1989) Domestication and spread of the cultivated rice In D.R. Harris and G.C. Hillman (Eds.). In Foraging and farming the evaluation of plant exploration. London: Unwin Hyman.
- Chourasia M, Patra BC, Parida M, Prasad SM, Sethy S, Sarangi DR, Mohanta RK, Katara JL and Samantaray S (2017) Inventorising the traditional rice germplasm in Cuttack district after five decades of green revolution. Indian Journal of Traditional Knowledge 16(4): 674-681.
- Dowswell CR, Paliwal RL and Cantrell RP (1996) Maize in the Third World. West view Press, Boulder, USA.
- Jambhulkar N, Patra BC, Reddy JN and Sarkar RK (2018) Developing mini core of rice germplasm for submergence tolerance. Indian Journal of Plant Genetic Resource 31(1): 139-146.
- Govindaswami S and Krishnamurty A (1959) Genetic variability among cultivated rice of Jeypore tract and its utility in rice breeding. Rice News Teller 7: 12-15.
- Green JM, Barker JA, Marshall EP, Froud-Williams RJ, Peters NB and Arnold GM (2001) Microsatellite analysis of the inbreeding grass weed Barren Brome (*Anisantha sterilis*) reveals genetic diversity at the within- and between-farm scales. Mol. Ecol. 10: 1035– 1045.
- Jennings PR (1966) Evaluation of partial sterility in *indica* x *japonica* rice hybrids. Bulletin No.5. IRRI, Philippines.
- Khush GS (1997) Origin, dispersal, cultivation and variation of rice. Plant Mol. Biol. 35(1-2): 25-34.
- Londo J, Chiang Y, Hung K, Chiang Y and Schall B (2006) Phytogeography of Asian wild rice, Oryza rufipogon, reveals multiple independent domestications of cultivated rice, Oryza sativa. Proc. of Natl. Acad. of Sci. 103: 9578-9583.
- Nachimuthu VV, Muthurajan R, Duraialaguraja S, Sivakami R, Pandian BA, Ponniah G, Gunasekaran, Swaminathan M, Suji KK and Sabariappan R (2015) Analysis of population structure and genetic diversity in rice germplasm using SSR markers: An initiative towards association mapping of agronomic traits in *Oryza sativa*. Rice 8: 30.
- Ngangkham U, Katara, JL, Parida M, Mohanty M, Parameswaran C, Kumar A, Patra BC, Singh ON and Samantaray S (2016). Assessment of Genetic Diversity of Weedy Rice (*Oryza sativa* f. spontanea) in Relation to Rice Relatives of Odisha, India. Intl. J. of Agri. Sci. 8(53): 2631-2637.
- Oka HI (1988) Origin of cultivated rice. Elsevier Publishing Company. Amsterdam.
- Samal R, Roy PS, Sahoo A, Kar MK, Patra BC, Marndi BC and Rao GJN (2018) Morphological and molecular dissection of wild rices from eastern India suggests distinct speciation between O. rufipogon and O. nivara populations. Nature Scientific Reports 8(2773): 1-13.



# Quality Seed Production and Maintenance Breeding for Enhancing Rice Yield

RK Sahu, RP Sah, P Sanghamitra, RL Verma, NKB Patil, M Jena, AK Mukherjee, MK Bag and ON Singh

## **SUMMARY**

Indian seed production system is a robust route to mitigate the seed requirement of the country. The seed class involves Nucleus, Breeder, Foundation and Certified seed with different seed quality standard at different levels to safe-guard the production of large quantity of quality seed for sustainable agriculture. The maintenance breeding is a mandatory step for the institute who are involved in development of variety. The developer maintains the seed purity of released varieties by curbing the chance of out crossing and genetic drift. The quality seed is the first and prime requisite for grain production, which alone contribute about 30% of yield improvement. Further, seed traits such as seed dormancy, viability, priming, foliar spray etc. are being given importance to improve cultivars for seed traits. Thus, it is important to deliver a healthy, improved variety seed to meet the seed requirement of the country and to dissect the seed traits for development of cultivar to cope with changing climate. Availability of good quality seed at the right time wherever it is needed with agreeable price, very much plays a major role in the highest grain production of a nation. The Indian seed delivery system which is backed by both formal and informal seed system has a good structural network for sufficient availability of seed but, the seed replacement rate and the varietal replacement rate are under desirable limit; majority of seed requirement of our farmer is fulfilled by informal seed system is one of the major factor responsible for this. Gaps in seed systems which include non-availability of many high yielding varieties in the seed chain, non-availability of sufficient quantity of quality seed, deterioration in seed quality, long time span for seed quality testing and non-assurance of genetic purity of Marker Assisted Selection developed varieties. Possible solutions for different constraints to strengthen the seed system has been discussed in the Chapter.

## **1. INTRODUCTION**

Seed is an enigmatic genetic capsule essential for multiplication and establishment of species from one generation to another. It is a fertilized ovule containing the plant embryo, a unit of reproduction of a flowering plant, which is capable of developing into another true-to-type such plant. Rice crop is a monocot; seed propagating, either annual or perennial; hollow internode, with tillering habit and the apex bearing the panicle. The rice seed is caryopses, comprising of embryo and endosperm. The seed surface contains several thin layers of differentiated tissues that enclose the embryo and endosperm. The palea, lemmas, and rachilla constitute the hull in Indica rice but



in Japonica the hull usually includes rudimentary glumes and perhaps a portion of the pedicel.

Pure seed is the basic and important input for healthy crops and good production. Seed should be pure, free from other contaminants, and should fit within minimum seed standard as recommended. For this purpose, a seed production system in India recognizes different class of seed viz., Nucleus, Breeder, Foundation and Certified seed with different seed quality standard to safe-guard the quality of large quantity seeds of Indian farmers. The maintenance of high quality seed of a variety is referred as 'Maintenance breeding', where a breeder is maintaining the seed purity of a released variety when it undergoes production year after year. This involves maintaining morphological, physical and genetic purity of a variety for a long period of time. These efforts were highly successful in improving seed quality by curbing the chance of out crossing and genetic drift. Further, to exploit the potential yield of a rice variety, various biochemical, physiological, and management aspects were viewed under seed technological research programme. Therefore, seed traits such as, seed dormancy, viability, priming, foliar spray etc. are being given importance by the researchers to improve the cultivars. Thus, rice seed production and molecular dissection are now the researchable areas to meet the seed requirement of the country and development of cultivar to cope with changing climate. This chapter emphasizes the status of seed production in rice, seed research and way for minimizing constraints to safe-guard national seed security. The objective of the chapter is to highlight the status of (i) breeder seeds as indented by DAC-GoI, States Government, and other organizations of India, (ii)status of seed research (iii) methods and procedure involved in quality rice seed production, and (iv) constraints involved and mitigation for seed production and research

## 2. IMPORTANCE OF QUALITY SEED

Seed is the first input of agricultural production on which the performance and efficacy of other inputs depend. Good quality of seeds can contribute upto 30% increase in productivity (Hasanuzzaman2015). "Good seed harvests good crop", a good seed means a seed lot that adheres to all the parameters of minimum seed standard; this seed is generally termed as quality seed. A good quality rice seed should be pure, full and uniform in size, free from weeds, insect, disease and other inert matters and more over it should be viable (>80% germination).

Timely availability of good quality seed as per the requirement plays a major role in the higher grain production of a nation. In India, 75% small and marginal farmers are lagging behind in agriculture due to unavailability of resources or inputs including seed. Therefore, a strong and vibrant seed production and supply system is indispensable for food security of the country and accelerating growth in agriculture. Seed is the highest prioritized input in agriculture, on which agriculture sustains. Over past 70 years, improvement in seed system was targeted to secure the seed quality, accessibility and availability.



A conscious thought on quality seed surfaced in1886 when channelized seed production began with the establishment of Swedish seed association. The association was mainly involved in production and distribution of quality seeds of forage crop varieties. Later near about 19<sup>th</sup> century, Dr. E Helve established a seed testing laboratory in Denmark for seed testing and certification. Canadian scientist Dr. JW Robertson in proposed the production of foundation seed in 1917. In 1919 an International Crop Improvement Association (ICIA) was formed to overlook the development of procedure and standards for quality seed production and seed certification. However, the organization was later named as Association of Seed Certification Agencies (AOSCA 1969). The ICIA in 1946 defined 4 classes of seed in forage crops, which was also adopted for other grain crops in 1968.

In India, Department of Agriculture of Uttar Pradesh state produced and distributed 150 tons of wheat seed in 1900. During that period limited seed testing facility was available at Kanpur. Later, in 1920 Government of Uttar Pradesh emphasized the production and distribution of quality seed and initiated project for establishment of seed godown in every subdivisions/tehsil. Later the Royal Commission on Agriculture reviewed the production and distribution of seed in India in 1925. In 1945 private seed company entered the seed scenario (like Sutton's for temperate vegetables) and in 1946 All India Seed Producer's Association (AISPA) was formed by private seed growers. A report of Famine Enquiry Commission (1945) and Grow-More Food Program Committee (1952) emphasized that there was a need to multiply and distribute the quality seeds of improved varieties. So, in 2<sup>nd</sup> Five Year Plan (1956–61) a shape for India's formal seed system was designed with special emphasis on production of nucleus and breeder seeds, which were used in multiplication of further class of seeds.

## 3. IMPETUS FOR QUALITY SEED PRODUCTION

The important developments relating to seed sector in the country are highlighted below in the Table 1.

In 1966-67 the seed production programme for wheat and maize was started and after a year (1967-68) rice crop was also included. After a huge review and recommendation, on 2<sup>nd</sup> October 1969 Indian Seed Act has been in force in India. Indian Seed Act, 1966 is an act to provide measures for regulating the quality of certain seeds for sale and for matters connected therewith. Some highlights of this act are (i) constitution of Central Seed Committee by Govt. of India to advice Central and State Governments regarding the Act., (ii) establishment of Central Seed Laboratory, (iii) establishment of State Seed Lab for seed quality analysis, (iv) provision of notification of varieties by Govt. of India, (v) minimum limits of germination and purity of seeds and compulsory label fixing, (vi) notified seed standard fixed, (vii) identifiable as seed of the variety it claims, (viii) must have minimum prescribed purity & germination, (ix) seed container must bear labels containing correct particulars of the seed, (x) establishment of Seed Certification Agency, (xi) establishment of Central



Year	Event	Objective/s
1952	A standing experts committee on seeds was appointed by Indian Council of Agricultural Research (ICAR).	The committee formulated a programme structure to strengthen the seed production & distribution system under which Central Govt. provided financial assistance to the states.
1956-57	State Seed Farm Project was initiated	Different states started producing foundation seeds in State Seed Farms.
1957-1965	All-India Coordinated Project for maize, wheat, pearl millets and barley	This involved production of foundation and certified seeds.
1959	An agricultural production team, by Dr. Johnson was formed	To bring uniform standards of seed certification, seed laws and establishment of Seed Testing Lab for each States.
	Planning Commission appointed a Seed Multiplication Team	To review the various aspects of seed programmes.
1960	ICAR set up a Committee	To suggest ways for developing a strong seed production programme. The Committee recommended for establishment of Central & State agencies for the production of foundation seed and an independent seed certification agencies to safe-guard the quality seed. The same committee also recommended for enactment of National Seed Act and formation of agencies for enforcement of seed act.
1963	ICAR constituted a committee	National Seeds Corporation was established and Indian Seed Act was enacted in 1966
1964	A rapid varietal release systems for improved variety	The State Variety Release Committees (SVRC) was established

#### Table 1. Milestones in the development of Indian seed sector.

Seed Certification Board to advise the Govt. of India and State Govt. on all matters relating to certification, (xii) appointment of Seed Analyst for seed analysis in State Seed Laboratory, (xiii) appointment of Seed Inspector to collect seed samples of notified kind being offered for sale for analysis, and (xiv) forfeiture of property (seeds) belonging to any person convicted under this act due to contravention of the procedures under this act.

Further, first turning point in shaping an organized seed industry was through National Seed Project (NSP) Phase-I (1977-78) which initiated the establishment of State Farms Corporation of India (SFCI), 4 State Seeds Development Corporations (SSDCs) and Breeder Seed Production (BSP) units. In the Phase-II of NSP (1985)13



additional SSDCs and 19 state seed certification agencies were established for quality seed production. After 10 years (1988-89) a New Seed Development Policy was formulated which gave access to the private individuals with strong R&D base for product development.

To achieve the food grain demand in future, it was felt that the Seed Replacement Rate (SRR) of various crops needs to be enhanced. This would require a major increase in the production of quality seeds with the involvement of both public and private sector. To safeguard the interests of Indian farmers and agro-biodiversity conservation, and to guard the exploitation of farmers by unscrupulous elements, the National Seed Policy (2002), a regulatory system, was formed. Later for regulating the production, distribution, quality of seeds for sale, import, export and to facilitate production and supply of seeds of quality and for matters connected therewith or incidental thereto, a seed bill (2004) was proposed. The government has proposed new amendments to the bill in April 2010 and November 2010, accepting most of the recommendations given by the Standing Committee. Few highlights of the Seed Bill (2004) are (i) all varieties of seeds for sale have to be registered, (ii) the seeds are required to meet minimum standards, (iii) transgenic varieties only be registered after clearance certificate as per the Environment (Protection) Act, 1986, (iv) exemption of farmers from the requirement of compulsory registration (v) farmers are allowed to sow, exchange or sell their own seed and planting material without any formalities required by registered seeds but, farmers cannot sell seed under a brand name, and (vi) provision for claim of compensation in case a registered variety of seed fails to perform to expected standards.

## 4. REGULATION OF SEED SYSTEM

The national seed requirement is taken care of through formal seed system (FSS) and informal seed system (ISS). Formal seed system is characterized by large scale production of seed of officially released varieties with strict quality assurance mechanism. This system is well organized and systematic, usually starts with development of different types of varieties/hybrids. The principles in the FSS are to maintain varietal identity, purity and to produce seed of optimal physical, physiological and sanitary quality (Reddy et al. 2007). Formal seed system is managed by Government body (Government Institutions, State Government Farms, University farms & KVKs) and registered seed growers (NGOs, Private Companies) whereas ISS is managed by farmers and sometimes private seed growers.

Varietal deterioration may happen with the repeated multiplication of the same variety year after year. This deterioration accommodates mixture of seeds, undesirable pollination or outcrossing, occasional mutation and genetic drift. This overall affects varietal genetic purity and crop performance. This deterioration is taken care of in the FSS through production of Nucleus, Breeder, Foundation and certified seed; but in ISS it is not well guarded. Therefore, it is required to create awareness among the farmers/seed growers to produce quality seed in their field for their own use.



It is reported that more than 85% of the total seed sown in India is produced by farmers themselves where quality seed constituted only 12% of the total seed sown each year (Reddy etal.2007) which is responsible for reduction in10-20% of yield.

# 5. CONSTRAINTS IN SEED PRODUCTION AND SEED RESEARCH

Indian seed production and supply system involves both Government institution and private sector including many collaborative ventures. A huge institutional framework is working for quality seed production and its distribution. Despite a healthier seed supply channel, continuous supply of good quality seeds remains as a problem from the seed producer to farmers. Therefore, farmers prefer to rely on their farm saved seeds which limits the SRR below 20% in many states. Besides, the variety replacement rate (VRR) is another section for maintaining the higher contribution in production through quality seed. More than 900 high-yielding varieties and hybrids of rice have been released for commercial cultivation, but about 318 are in the active seed production chain. The constraints involved in seed production and distribution are; seed purchase from unreliable sources, deterioration of seed quality when multiplied for long duration, unavailability of quality seeds, lower SRR or VRR, unawareness for method of seed production, time consuming seed quality testing, and sometimes non-assurance of genetic purity of MAS developed varieties. Further, the research on improvement on seed setting, seed dormancy, seed viability, seed and seedling vigour is very little which restrict varietal features and its adoption by farmer

# 6. DEVELOPMENTS IN SEED SYSTEM AND SEED RESEARCH

A rationalized system for breeder seed production programme is taken up by the Indian Council of Agricultural Research (ICAR) Institutes and State Agricultural Universities (SAUs). However, certified/quality seed production programme is taken care by the National Seeds Corporation Ltd. (NSC), the State Seeds Corporations, the State Department of Agriculture, State Seed Farms, State Agricultural Universities Farms, Krishak Bharati Cooperative Ltd. (KRIBHCO), Private Seed Farms etc. to ensure quality seeds supply to farmers.

The Indian seed production programme passes through 3-4 generations of seed multiplication in a phased manner. The system provides an adequate safeguard for seed quality assurance during multiplication to maintain the purity of the variety as it flows from the nucleus seed to the seed for farmer (Certified or TL seeds). A large number of seed companies and producers are being engaged in seed channel. To regularize the system and to monitor the quality seeds produced, about 15 State Seeds Corporation, 2 National level seeds Corporations, 34 State Departments of Agriculture, 21 Seed certification agencies, 94 Seed testing laboratories, many ICAR Institutes and State Agricultural Universities are jointly working in the seed platform.



# 7. SEED MULTIPLICATION CHAIN OF INDIA

Once a variety is released and notified it can be included in seed chain. The chain of seed production is presented below-

Farmer (Variety wise requirements) ↓ DAO (District Agriculture Officers) (Collected the data on variety demand) ↓ DDA (Deputy Director Agriculture) (Assessment of varietal demand, crop situation demand) ↓ Director Agriculture (actual indent to be placed, new variety to be introduced) ↓ DAC (Dept. of Agriculture & Cooperation) (National Indent of seed) ↓ Breeder seed producing organization (Produce the indented quantity of breeder seeds) ↓ SSC & Director Agriculture (lifting of breeder seeds and putting in seed chain) ↓ DAO (Organization of seed distribution) ↓ Farmer (Growers)

Responsibilities of the organization that takes up seed production (as per the class of seed) and the certification norms is presented below in Table 2.

Class of seed	Institutes/Organization/Agencies	Supervision	Certification
Nucleus seed	Developer, breeder, parent institutes	Breeder, developers	No need, responsibility of parent institution or developers
Breeder Seed	Developer, breeder, parent institutes, registered organization	Breeder, developers	Members assigned by seed certification agencies
Foundation Seed	Central Government agencies, State Departments, Agriculture Universities, State Farms, Private seed companies, Farmers producer organization	Concern producer	Members assigned by seed certification agencies
Certified Seed	Central Government agencies, State Departments, Private seed companies, Farmers producer organization, Agriculture Universities, State Farms	Concern producer	Members assigned by seed certification agencies
TL Seed	Any organization and farmers	Concern producer	No need, responsibility of producer

Table 2.Seed class and institution involved in seed production.



The breeder seed production status of the last five years revealed that the rice breeder seed producing organizations have produced more than the quantity of seed demanded every year (Table3).

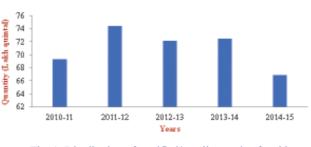
Year	Indent (q)	Production (q)
2012-13	5267	11455
2013-14	4837	10586
2014-15	4286	7757
2015-16	5026	5449
2016-17	5119	8765

Table 3. Trends in indent and production of breeder seed of rice (Chauhan et al.2017).

More than 300 varieties are under seed chain but, few varieties had highest indent among them. The year wise top five varieties of last five years were presented below (Table 4). Among these varieties Swarna, Cottondora Sannalu, IR-64, Mahamaya and Vijetha were released long years back and Sahabhagidhan, Swarna Sub-1 and Naveen were released recently. The old varieties are still under demand, which may be due to its higher adaptability, buffering capacity, consistent performance and also higher tolerance to stresses.

S. No.	Variety	2012-13	2013-14	2014-15	2015-16	2016-17
1.	Swarna (MTU 7029),	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	
2.	Cottondora Sannalu (MTU 1010)	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
3.	IR-64	$\checkmark$	$\checkmark$			$\checkmark$
4.	Mahamaya	$\checkmark$				
5.	Vijetha (MTU 1001)	$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$	
6.	Sahabhagidhan		$\checkmark$	$\checkmark$	$\checkmark$	$\checkmark$
7.	Swarna Sub-1			$\checkmark$	$\checkmark$	$\checkmark$
8.	Naveen (CR-749-20-2)					$\checkmark$
	ution of top most 5 varieties to dent (%)	27.01	30.9	34.8	28.6	31.2

From 2010 to 2015 about 355.18 lakh quintal quality rice seed was supplied, which was an average of 71.03 lakh quintal per year. The year wise production chart is presented in Fig. 1.







The Government of India periodically assesses the requirement and availability of seeds through State Governments and seed producing agencies in the bi-annual zonal seed review meetings and the national *kharif* and *rabi* meet. The DAC&FW facilitates the seed producing agencies to ensure the requirement of seeds to the maximum extent possible.

# 8. MOLECULAR RESEARCH IN SEED AND RELATED TRAITS

The important traits included under seed traits are seed dormancy, anaerobic germination, seed shattering, seed longevity, seed viability, seed weight and seed vigor. Phenotypic characterization on these traits has been reported for lot of genotypes. However, molecular studies on these traits are very scanty. Few QTLs and genes were identified for seed traits.

Seed dormancy is the failure in germination of mature and viable seeds under favourable condition. Generally the cultivated species are having no or short period of dormancy than the non-cultivated ones. The phenomena may be seen in either way i.e. weak dormancy promotes a uniform germination, whereas high dormancy prevents pre-harvest sprouting but inhibits germination and reduce seed quality. Thus, moderate dormancy levels (15-20 days) would be desirable. The dormancy lasts for few days to more than a month. This trait is controlled by both environmental as well as genetic factors inhabit in both maternal and embryonic tissues. Seed dormancy is genetically controlled by the genotypes of both the mother plant and the embryo. The maternal plant form tissues surrounding the embryo, such as the seed coat (testa) which creates barriers to radicle growth on imbibition. This "coat-imposed" dormancy depends on the anatomy of the seed. In rice, qSD7-1, a clustered QTL (qSD7-1/qPC7) was delimited to the pleiotropic Rc locus and found to control seed dormancy by regulating ABA biosynthetic pathway in rice. Moreover Sdr4, a global regulator of seed maturation was cloned in rice and was positively regulated by OsVP1 (Sugimoto et al 2010). The QTL mapped in the 12 chromosomes of rice except chromosome 10 included clusters QTL such as gSD7/gPC7, gSD1-2/gPH1 and gSD7-2/qPH7 (Ye et al. 2013).Further, soil flooding is one of the abiotic constraints in the rainfed lowland areas. Starchy seeds were shown to be especially tolerant of anaerobiosis because they are able to maintain a high energy metabolism under oxygen deficiency when compared with fatty seeds. The calcineurin-interacting protein kinase (CIPK15) gene was reported that signals pathway that regulates RAmy3D, which affects the expression of coleoptiles in anoxic conditions and anaerobic germination of rice. The first natural variant of QTL qAG-9-2 that enhances anaerobic germination was reported and fine-mapped to OsTPP7, which is a gene encoding a trehalose-6-phosphate phosphatase (Kretzschmar et al. 2015). Two major QTLs for anaerobic germination viz., AG1 and AG2 were identified (Angaji et al. 2010). Researchers has also identified candidate proteins/genes for improving seed vigour in rice plants such as OsHSP18.2 (Kaur et al. 2015); OsALDH7, ACCase, PI3K (Liu et al. 2012); OsLOX (Wang et al. 2008). Only these few traits have been studied at



molecular level and much closed gene were reported for seed related traits till date, which creates a huge gap in seed research.

# 9. PROCESS AND PROGRESS AT ICAR-NRRI ON SEED PRODUCTION AND RESEARCH

ICAR-NRRI is producing a large quantity of Breeder seed and Truthfully Labeled (TL) seed as per the indent of Department of Agriculture and Cooperation (DAC), Government of India, State Governments, other organizations and requirement of farmers. The institute is one of the volunteer centers for rice breeder seed production under ICAR. The AICRP-NSP (Crops) under ICAR-Indian Institute of Seed Science (IISS), Mau, Uttar Pradesh (the coordinating center) looks after the indent / allotment of seed production decided by DAC, Government of India and also facilitates monitoring of the seed production plots. The information from indent of the seed to the production and final lifting or sale is being documented in the form of BSP-I, BSP-II, BSP-III, BSP-IV and BSP-V. The BSP-I depicts the variety-wise requirement of Breeder Seed as per the Indent of Department of Agriculture Cooperation & Family Welfare compilation, BSP-II elaborates the variety-wise area of production and time of monitoring of Breeder Seed production plots, BSP-III includes State Monitoring Report for certification, BSP IV reports on quantity of Breeder Seed Produced (Actual Seed Production during rabi and kharif season) and BSP-V elucidates the lifting and non-lifting status of Breeder seed by indenter (center wise).

Breeder seed plots of the institute is monitored by a Central team constituted by ICAR-IISS, Mau to inspect the crop condition and field level purity; and also by a state level monitoring team for seed certification. The state level monitoring team includes breeder from NRRI, representatives of Odisha State Seed and Organic Product Certification Agency (OSSOPCA), State Agricultural University, Odisha State Seed Corporation (OSSC) and National Seed Corporation (NSC). The production of breeder seed is again reviewed by ICAR-DAC in the annual breeder seed review meeting.

Being a leading research institute of rice, ICAR-NRRI is supplying high quality breeder seeds to governments and other agencies to produce highest quality Foundation and Certified seeds for the country. During last 5 years the institute has produced about 3383.34 q breeder seed against the indent of 3333.50 q (Table 5).

Besides nucleus and breeder seeds, ICAR-NRRI has also been producing TL seed under Participatory Seed Production (PSP) programme where TL seed is produced in the farmer's field with inputs of the farmers and technical know-how and supervision of the NRRI scientists. This programme was initiated 5 years back and more than 3400 q seed of mega varieties viz. Swarna Sub-1, Pooja, Naveen and Sarala was produced.

The institute is also imparting training on quality seed production, management and storage, where farmers, state government officials, representative of various NGO's and seed producers were successfully trained during these last years.

Seed research was also a priority area along with seed production at our institute. The ICAR-NRRI has characterized the released varieties of the institute for days to



Table 5. Breeder seed indent and production of rice at NRRI during 2012-13 to
2016-17.

	DAC	No. of	Production	
Year	Indent	varieties	(q)	Mega indented varieties
2012-13	571	25	651.35	Swarna Sub 1, Pooja, Shatabdi, Naveen, Sarala
2013-14	481	49	483.12	Swarna Sub1, Pooja, Naveen, Shatabdi, Sarala
2014-15	622	44	607.27	Swarna Sub 1, Pooja, Naveen, Shatabdi, CR 1014
2015-16	747	62	768.70	Swarna sub 1, Naveen, Varshadhan, CR Dhan 601, CR Dhan 501
2016-17	912.5	43	872.90	Swarna Sub 1, Naveen, CR Dhan 500, Shatabdi, Varshadhan,
Total	3333.5		3383.34	

seed dormancy and duration of viability which was depicted in NRRI Annual Report 2016-17. The seedling vigour of rice has direct relevance with antioxidant and amylose content in seed of pigmented rice. The pigmented rice are rich in genes for seed traits and are good source for identification of donor. The seed and seedling vigour are important traits especially for aerobic condition (Kumar et al. 2016). The molecular study for the seed traits has been initiated to find out the relevant markers to start breeding for seed traits and development of essentially derived varieties.

# **10. SEED MULTIPLICATION SYSTEM OF INDIA**

Once a variety is released, it is the responsibility of the parent Institute to safeguard the seed quality of that particular variety and to make available the indented quantity of Breeder seed of the variety in the seed chain. Maintenance breeding and production of Nucleus seed safe-guards the quality of the variety at Institute level and this Nucleus seed is used as basic seed for production of Breeder seed which cares for the quality of the National seed chain.

Indian seed system is a robust and full proof one that strictly adheres to three generation system (Breeder seed $\rightarrow$ Foundation seed $\rightarrow$ Certified seed); but in exigencies four or five generation model is followed where foundation seed stage II or certified seed stage II is produced.

The nucleus seed plots are planted in paired rows, each paired row contains plants from one single selected panicle. All around the plots 8 rows border line of the same variety (from bulk breeder seed) is transplanted. If any off-type plants are observed in any of the panicle progeny row, then that particular paired row is totally discarded/rogued-out. If any off-type plant with different grain type is marked (obviously observed after flowering) then the panicle progeny rows where the off-type is observed and the adjacent progeny rows (at both sides) are discarded to restrict chance pollination involving the off-type plant. After thorough roguing, sufficient true-to-type panicles (at least 500) are selected based on the morphological identity, uniformity and genetic purity to maintain nucleus seed for next generation.



The border row is supposed to restrict the foreign pollen flow and is not considered as seed. The panicle progeny rows are harvested and threshed separately; passes through table-top examination and later bulked as nucleus seed. This Nucleus seed is used for production of Breeder Seed.

Breeder seed (BS) is the progeny of nucleus seed, where, after every 6-8 rows of planting a skip row is allowed to facilitate intercultural operations and proper roguing. Here also 8 row borders all around the plot is maintained which is not considered as seed during harvest. Off-type plants if observed are simply rogued out. The crop is monitored by Central and State monitoring team. The genetic purity of breeder seed should be maintained at 100 percent. Breeder seed tag is golden yellow in colour and size is 12x6 cm.

Foundation seed is the progeny of breeder seed, called foundation seed stage I; and when foundation seed is the progeny of foundation seed, it is called foundation seed stage II. The foundation seed stage I is used for the production of foundation seed stage II. The minimum seed standard for Foundation seed stage I and stage II are same. Production of Foundation seed stage II is undertaken only when it is expressed by the seed certifying agencies that breeder seed is in short supply and stage II foundation seed has to be produced to meet the seed demand. Foundation seed is monitored by the state certifying agency. The genetic purity of foundation seed should be maintained at 99.5 percent. Foundation seed tag is white in colour and size is 15x7.5 cm.

Certified seed is the progeny of foundation seed I or II. But, certified seed can also be the progeny of certified seed provided this reproduction does not exceed three generations beyond foundation seed stage I. Certified seed produced from foundation seed is called certified seed stage I, while Certified seed produced from certified seed is called certified seed stage II. Certified seed is monitored by the state certifying agency and its genetic purity is 99%. Certified seed tag is blue in colour and size is15x7.5cm.

One additional class of seed is produced and marketed in India, known as Truthfully labeled seed (TL Seed) where certification is not required but, minimum seed standard must be fulfilled. It is applicable to both notified varieties and variety developed by any person or agency. Seed inspectors are the persons who can guard the quality of seed which is on sale. So the quality of marketed TL seed can be inspected by them if doubt on quality arises; and if it fails the quality test, the sale of that seed can be stopped. The tag colour of TL seed is opel green and size is 15x10 cm.

## **11. KNOWLEDGE GAPS**

Non-availability of many high yielding varieties in seed chain: A large number of high yielding varieties (HYVs) suitable for different agro-climatic situations are released in India. But, many high yielding varieties are not under seed chain may be due to lack of popularization. Therefore, it is required to include these HYVs under front line demonstration (FLD) programmes for popularization among the



farmers; and information regarding these varieties need to be communicated to all the officials working under seed production and marketing for there further popularization.

- Insufficient quantity of quality seed: Available quality seed of improved rice varieties and hybrids is many times inadequate due to climatic disturbance, improper technology, wrong handling etc. and is considered as one of the major constraints for higher productivity. This problem may be due to the fact that (i) presently, high volume-low quality seeds are available with the farmers and low volume-high quality seeds are mostly available with the public sector, (ii) non-lifting of produced breeder seed by Government Institutions and private agencies, which hamper the production of sufficient quantity foundation and certified seed. These problems can be sorted out by (a) engagement of more officials for monitoring of quality seed production, (b) involvement of more volunteer agencies such as village-based/community based seed banks to take up the Foundation and Certified seed production programme and (c) designing a policy framework for advance payment of indented quantity of seeds to limit the problem of non-lifting.
- Seed quality deterioration: Seed is a biologically living entity, whose quality deteriorates if the minimum standard for seed production and storage steps are not followed. Varietal deterioration occurs due to repeated multiplication of the same varieties year after year probably due to undesirable pollination or outcrossing, occasional mutation and genetic drift. The formal seed system guards against the deterioration of seed quality through production of Nucleus, Breeder, Foundation and Certified seed, the quality of which is well monitored. But due to non-availability of this quality seed, large number of farmers depend on their farm-saved seeds or TL seeds from the market where quality parameters are not well-guarded. So these seeds used by the farmers show presence of seed of other varieties, mixtures, impurities and less germination percentage that affects the total grain production. This seed quality deterioration can be checked through; (i) Awareness generation among seed growers and farmers regarding quality seed and (ii) Imparting training on Quality Seed production technology to the seed growers and farmers.
- Long time span for seed quality testing: Grow-out-test (GOT) is a procedure to test the genetic purity of the seed. It involves assessing the several morphological characteristics in different developmental stages which takes a long time span, almost the entire cropping season. Furthermore, GOT is a simple way to analyze the genetic purity based on the basis of visual detection which can be easily affected by growing conditions. To make it more exact and to reduce the time span, DNA based testing will be a proven alternative for GOT.
- Non assurance of genetic purity of MAS developed varieties: The present era of molecular breeding has now accelerating gene introgression in existing varieties resulting in release of varieties like Improved Tapaswini, Improved Lalat, Swarna-Sub1 etc., which are now under seed chain. Most of these MAS developed varieties



are quite similar with parents except the introgressed genes. Here, selection of true to type plants of varieties developed through MAS (where no distinct phenotypical difference) can only be possible through molecular level detection or though DNA fingerprinting. This molecular marker based genetic purity testing (MGPT) at nucleus seed level will provide 100% purity and high level of seed purity on subsequent class of seed.

- Awareness and training: Intermediaries seed producers involved in production and distribution of seeds have a large contribution in supply of paddy seeds to the farmers. The question arises whether all these seeds are quality seeds? The report says, unawareness in many producers regarding quality seed production procedure leads to poor quality control. Intensive training to trainers and seed producers about seed production, quality management and purity testing will help in increasing the high volume quality seed.
- Seed technology research: Characterization of released varieties for seed traits like seed viability duration or longevity and seed dormancy is poorly documented. These information are always needed by the producers and farmers for better seed multiplication, proper time of harvesting and safe storage. There are various theories reported for genetic basis and physiological basis of seed related traits. But, utilization of these information is very poor. A strong platform for seed research can be a base to utilize the existing information and dissecting the molecular, proteomics and metabolomics basis on expression of seed related traits. The advancement of new molecular technology will explore different seed traits which will lead to seed trait specific breeding to improve the cultivar performance such as improvement of cultivar for capacity to germinate under anaerobic condition, no seed shattering, prolonged seed longevity, intermediate seed dormancy, high seed viability, appropriate seed dimension, higher seed weight, seed pigmentation if necessary, improve seed coat permeability etc. Introgressions of these traits are mostly relevant for high seed vigor, seed storage and optimum plant stand in field.

## **12. WAY FORWARD**

Indian seed delivery system for farmers has a good structural network for sufficient availability of seed. But, the SRR and VRR are still under desirable limit. Many points have been discussed in this chapter related to production, management and research options for safe guarding the seed quality and to speed up the process of deliveries. QTLs have been identified but for seed traits, only few candidate and functional genes are known till date. The emphasis and initiatives now need to be made for (i) designing policy framework for timely lifting of seed, (ii) involvement of more officials for proper monitoring to produce quality seed, (iii) authorization to national level institute for issuing certification for seed production plot; this will be helpful to cover more seed plot area for certification, (iv) mapping and development of marker for introgression of seed traits, (v) involvement of molecular tools for rapid purity testing procedure,(vi) advanced level capacity building of stake holders involved in seed



chain, (vii)creation of buffer stock of seed in form of seed bank at village level, and establishing seed hubs,(viii)development of a local seed system as an alternative to formal system (like 4S4R model developed by ICAR-NRRI, Cuttack). Once these points are taken care of, the quality seed production and distribution system will smoothen and the Country will achieve higher rice grain production.

#### References

- FAO (2017) Food and Agriculture Organization of the United Nations, Rome, Italy, 20(1), April 2017.
- Annual Report (2016-17) Department of Agriculture Cooperation and Farmers Welfare, Ministry of Agriculture and Farmers welfare, Government of India, Krishi Bhawan, New Delhi.
- Hasanuzzaman M (2015) Concept note. http://hasanuzzaman.weebly.com/uploads/9/3/4/0/9340 25/seed quality.
- Chauhan JS, Prasad Rajendra S, Pal Satinder and Choudhury PR (2017) Seed systems and Supply chain of rice in India. Journal of Rice Research 10(1): 9-16.
- Reddy Ch Ravinder, Tonapi VA, Bezkorowajnyj PG, Navi SS and Seetharama N (2007) Seed system innovations in the semi-arid tropics of Andhra Pradesh, International Livestock Research Institute (ILRI), ICRISAT, Patancheru, Andhra Pradesh, India, 224.
- Anonymous2016. Agricultural Statistics at a Glance (2015) Directorate of Economics & Statistics. Department of Agriculture, Cooperation and Farmers Welfare, Ministry of Agriculture & Farmers Welfare, Government of India, New Delhi, 479.
- Sugimoto Kazuhiko, Yoshinobu Takeuchi, Kaworu Ebana, Akio Miyao, Hirohiko Hirochika, Naho Hara, Kanako Ishiyama, Masatomo Kobayashi, Yoshinori Ban, Tsukaho Hattori, Masahiro Yano (2010) Molecular cloning of *Sdr4*, a regulator involved in seed dormancy and domestication of rice. Proceedings of the National Academy of Sciences USA107 (13): 5792–5797.
- Ye H, Beighley DH, Feng J, Gu XY (2013)Genetic and physiological characterization of two clusters of quantitative trait loci associated with seed dormancy and plant height in rice. G3 (Bethesda) 3:323–31.
- Kretzschmar T, Pelayo MAF, Trijatmiko KR, Gabunada LFM, Alam R, Jimenez R, Mendioro MS, Slamet-Loedin IH, Sreenivasulu N, Bailey-Serres J, Ismail AM, Mackill DJ, Septiningsih EM (2015) A trehalose-6-phosphate phosphatase enhances anaerobic germination tolerance in rice. Nature Plants: 15124.
- Angaji SA, Septiningsih EM, Mackill DJ and Ismail AM (2010) QTLs associated with tolerance of anaerobic conditions during germination in rice (Oryza sativa L.). Euphytica 172:159 -168.
- Kaur H, Petla BP, Kamble NU, Singh A, Rao V, Salvi P, et al. (2015) Differentially expressed seed aging responsive heat shock protein OsHSP18.2 implicates in seed vigor, longevity and improves germination and seedling establishment under abiotic stress. Frontiers in Plant Science 6:71.
- Kumar R, Kumawat N, Kumar S, Kumar R, Kumar M, Sah RP, Kumar U and A Kumar (2016) Direct Seeded Rice: Research Strategies and Opportunities for Water and Weed Management. Oryza 53(4): 354-365.
- Liu J, Zhou J, and Xing D (2012) Phosphatidylinositol 3-kinase plays a vital role in regulation of rice seed vigor via altering NADPH oxidase activity. PLoS ONE 7:e33817.
- Wang R, Shen WB, Liu L, Jiang L, Zhai H and Wan J (2008) Prokaryotic expression, purification and characterization of a novel rice seed lipoxygenase gene OsLOX1. Rice Science 15:88– 94.<sup>⊕</sup>



# Utilization of Cultivated and Wild Gene Pools of Rice for Resistance to Biotic Stresses

## MK Kar, LK Bose, M Chakraborti, M Azharudheen TP, S Ray, S Sarkar, SK Dash, JN Reddy, DR Pani, M Jena, AK Mukherjee, S Lenka, SD Mohapatra and NN Jambhulkar

### SUMMARY

Productivity of rice is often adversely affected by several biotic stresses. The major biotic stresses such as blast, bacterial blight, sheath blight, brown planthopper and yellow stem borer play crucial roles in reducing the productivity and quality of rice. Among the various control measures available for mitigating biotic stresses, host plant resistance is most effective, economic and eco-friendly. Wild and cultivated gene pools of rice are important sources for many resistance genes/QTLs, which are successfully utilized in resistance breeding programme. In this chapter, a comprehensive assessment of the use of wild and cultivated gene pools of rice for imparting resistance to major biotic stresses has been presented.

## **1. INTRODUCTION**

Like all other crop plants, rice (Oryza sativa) also suffers from several biotic and abiotic stresses that seriously affect its production. A wide range of pathogens, insects, nematodes and other pests attack the rice plant in different parts of the world. Magnitude and the type of damage caused by pests vary in different regions. Among them, diseases like blast, bacterial blight (BB) and sheath blight (ShB) and insects like brown planthopper (BPH) and yellow stem borer (YSB) are of major concern in India as well as many other parts of the world. Despite the availability of several control measures for mitigating pest damage in crop plants, developing cultivars tolerant to major insect-pests and diseases prevalent in an area is the easiest, most economic and most eco-friendly measure available to the farmers. At the same time, the system is highly dynamic in its nature due to continuous co-evolution of genes conferring resistance or susceptibility in hosts and their corresponding gene for virulence in pests. Genes conferring resistance are distributed across primary, secondary and tertiary gene pool of the crop. Judicious use of these genes and genetic resources to minimize losses caused by pests remains an important challenge for rice researchers worldwide

In India, systematic research efforts to impart host plant resistance in rice is undergoing from more than 70 years. The biotic stress breeding programme at the National Rice Research Institute, Cuttack, Odisha has evolved over time depending on the dynamic pest profile of the crop and advances in the technologies available. The institute was established in 1946 in the backdrop of the Bengal famine caused due to *Helminthosporium* leaf spot. Hence during the first two decades, the emphasis



was mainly given to developing brown spot resistant genotypes. Eventually, breeding for tolerance against blast and yellow stem borer (YSB) was also taken up. With the introduction of high yielding semi-dwarf varieties like TN 1 during early 60's, bacterial blight became a severe threat to rice production. The 70's and 80's saw the major focus being directed towards breeding for bacterial blight tolerance. With the outbreak of brown planthopper in the late 1970's, breeding for BPH tolerance has also taken a centre stage. Sheath blight, though very severe even during 1960's in countries like the Philippines, was not a stress capable of causing economic damage to the rice industry in India until recently. But the severe incidence of sheath blight is being reported of late especially in the most productive parts of the country like Punjab and even in many regions of Orissa where intensive farming is practiced to raise the crop.

The global and national efforts towards understanding the mechanism of resistance and developing cultivars with biotic stress tolerance against the five major rice pests, viz., blast, bacterial blight, sheath blight, brown planthopper and yellow stem borer have been reviewed in this chapter, with major emphasis being given to the work carried out at ICAR-NRRI, Cuttack.

# 2. RICE BLAST (MAGNAPORTHE ORYZAE) RESISTANCE

Rice blast disease caused by *Magnaporthe oryzae* is one of the most destructive disease causing huge losses to rice yield and thereby posing a great threat to world food security. Use of blast resistant cultivars is the most effective, economic and environmentally sustainable way of managing this pathogen. Till today more than 100 blast resistance genes have been identified (Table 1). Of these, 45% are from *japonica* cultivars, 51% from *indica* cultivars and the rest 4% are from wild species of rice. Blast resistance genes and their genetic location in different rice cultivars have been reviewed by Sharma et al. (2012). Recently, Liang et al. (2016) reported that *pi* 66(t) is one of the three recessive genes controlling rice blast, and is the first major gene for resistance to be mapped on chromosome 3. Li et al. (2017) identified a new gene from a rice variety Digu which is effective against broad spectrum of *M. oryzae* races. An exhaustive list of the reported blast resistance genes with their corresponding sources and their chromosomal locations have been mentioned in Table1.

Blast disease was first reported in India in 1913 and the first devastating epidemic due to rice blast was reported in 1919 in Tanjore delta. Since then several works were carried out in various parts of the country. An important gene for blast resistance, *Pi-kh* was identified from *indica* variety Tetep at ICAR-National Research Centre for Plant Biotechnology, New Delhi. They further characterized, fine mapped, cloned and functionally validated the resistance gene. The corresponding virulent gene, *AvrPi54* in the pathogen was also successfully cloned by the team, which contributed significantly in the detailed understanding of host-pathogen interaction (Ray et al. 2016).

Hittalmani et al. (2000) used closely linked RFLPs and polymerase chain reaction (PCR)-based markers to put three blast resistance genes *Pi1*, *Piz-5* and *Pita* into a



St. No.         Certe name         Location (Chr No)         Sources of resistance           1 $Mpiz$ 11         Zenith           2 $PbI$ 11         Modan           3 $PBR$ 11         St. No 1           4 $Pi(t)$ 4         P167           5 $PiI$ 11         LAC23           6 $PiI0$ 5         Tongil           7 $PiI1$ 8         Zhai-Ya-Quing8           8 $Pi12$ 12         K80-R-Hang, Jiao-Zhan, Moroberekan           9 $Pi13(t)$ 6 $O.minuta$ (W), Kasalath (I), Maowangu           10 $Pi14(t)$ 2         Maowangu           11 $Pi15(t)$ 12         Moroberekan           13 $Pi16(t)$ 2         Aus373           14 $Pi17$ 7         DJ123           15 $Pi18(t)$ 11         Suweon365           16 $Pi19(t)$ 12         Aichi Asahi           17 $Pi20$ 12         IR24           18 $pi21$ 4         Owarihatamochi	C1 N	0		с с : <i>с</i>
2 $PbI$ 11Modan3 $PBR$ 11St- No 14 $Pi(t)$ 4P1675 $PiI$ 11LAC236 $PiI0$ 5Tongil7 $PiI1$ 8Zhai-Ya-Quing88 $Pi12$ 12K80-R-Hang, Jiao-Zhan, Moroberekan9 $Pi13(t)$ 6 $O.$ minuta (W), Kasalath (I),Maowangu10 $Pi14(t)$ 2Maowangu11 $Pi15$ 9GA2512 $Pi15(t)$ 12Moroberekan13 $Pi16(t)$ 2Aus37314 $Pi17$ 7DJ12315 $Pi18(t)$ 11Suweon36516 $Pi19(t)$ 12IR2418 $pi21$ 4Owarihatamochi19 $Pi22(t)$ 6Gumei 223 $Pi25(t)$ 2IR624 $Pi26$ 6Gumei 225 $Pi26(t)$ 5Azucena26 $Pi27$ 1Q1427 $Pi27(t)$ 6IR6428 $Pi28(t)$ 10IR6429 $Pi3(t)$ 12IR6430 $Pi3(t)$ 6Pai-kan-tao31 $Pi30(t)$ 11IR6432 $Pi31(t)$ 12IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(t)$ 1Hokkai 18837 $Pi38$ 11Tadukan	Sl. No.	Gene name	Location (Chr No)	Sources of resistance
3 $PBR$ 11St- No 14 $Pi(t)$ 4 $P167$ 5 $PiI$ 11 $LAC23$ 6 $PiI0$ 5 $Tongil$ 7 $PiI1$ 8 $Zhai-Ya-Quing8$ 8 $Pi12$ 12 $K80-R-Hang, Jiao-Zhan, Moroberekan$ 9 $Pi13(t)$ 6 $O.\ minuta$ (W), Kasalath (I),Maowangu10 $Pi14(t)$ 2Maowangu11 $Pi15$ 9 $GA25$ 12 $Pi15(t)$ 12Moroberekan13 $Pi16(t)$ 2Aus37314 $Pi17$ 7DJ12315 $Pi18(t)$ 11Suweon36516 $Pi19(t)$ 12Aichi Asahi17 $Pi20$ 12IR2418 $pi21$ 4Owarihatamochi19 $Pi22(t)$ 6Suweon36521 $Pi23$ 5Suweon36521 $Pi25(t)$ 2IR624 $Pi26$ 6Gunnei 225 $Pi26(t)$ 5Azucena26 $Pi27$ 1Q1427 $Pi27(t)$ 6IR6428 $Pi28(t)$ 10IR6430 $Pi3(t)$ 6Pai-kan-tao31 $Pi30(t)$ 12IR6433 $Pi32(t)$ 12IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(t)$ 1Hokkai 18837 $Pi38$ 11Tadukan		•		
4 $Pi(t)$ 4P1675 $PiI$ 11LAC236 $PiI0$ 5Tongil7 $PiI1$ 8 $Zhai-Ya-Quing8$ 8 $PiI2$ 12K80-R-Hang, Jiao-Zhan, Moroberekan9 $PiI3(t)$ 6 $O.$ minuta (W), Kasalath (I), Maowangu10 $PiI4(t)$ 2Maowangu11 $PiI5$ 9 $GA25$ 12 $PiI5(t)$ 12Moroberekan13 $PiI6(t)$ 2Aus37314 $PiI7$ 7DJ12315 $PiI8(t)$ 11Suweon36516 $Pi19(t)$ 12Aichi Asahi17 $Pi20$ 12IR2418 $pi21$ 4Owarihatamochi19 $Pi22(t)$ 6Suweon36520 $Pi23$ 5Suweon36521 $Pi24(t)$ 1Azucena22 $Pi25$ 6Gumei 223 $Pi25(t)$ 2IR624 $Pi26$ 6Gumei 225 $Pi26(t)$ 5Azucena26 $Pi27$ 1Q1427 $Pi29(t)$ 8IR6430 $Pi3(t)$ 6Pai-kan-tao31 $Pi30(t)$ 12IR6433 $Pi32(t)$ 12IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(t)$ 1Hokkai 18837 $Pi36$ 8Q6138 $Pi37$ 1 <td></td> <td></td> <td></td> <td></td>				
5 $Pil$ 11LAC236 $Pil0$ 5Tongil7 $Pil1$ 8 $Zhai-Ya-Quing8$ 8 $Pil2$ 12 $K80.R-Hang, Jiao-Zhan, Moroberekan9Pil3(t)6O.minuta (W), Kasalath (I), Maowangu10Pil4(t)2Maowangu11Pil59GA2512Pil5(t)12Moroberekan13Pil6(t)2Aus37314Pil77DJ12315Pil8(t)11Suwcon36516Pil9(t)12Aichi Asahi17Pi2012IR2418pi214Owarihatamochi19Pi22(t)6Suwcon36520Pi235Suwcon36521Pi256Gumei 222Pi256Gumei 223Pi25(t)5Azucena26Pi271Q1427Pi27(t)6IR6428Pi28(t)10IR6429Pi29(t)8IR6430Pi3(t)12IR6431Pi30(t)11IR6432Pi31(t)12IR6433Pi32(t)12IR6434Pi338IR6435Pi3411Chubu3236Pi35(t)1Hokkai 18837Pi3811Tadukan$				
6 $Pi10$ 5Tongil7 $Pi11$ 8 $Zhai-Ya-Quing8$ 8 $Pi12$ 12 $K80-R-Hang, Jiao-Zhan, Moroberekan$ 9 $Pi13(i)$ 6 $O. minuta$ (W), Kasalath (I), Maowangu10 $Pi14(i)$ 2Maowangu11 $Pi15$ 9 $GA25$ 12 $Pi15(i)$ 12Moroberekan13 $Pi16(i)$ 2 $Aus373$ 14 $Pi17$ 7 $DJ123$ 15 $Pi18(i)$ 11Suweon36516 $Pi19(i)$ 12Aichi Asahi17 $Pi22$ $i$ $R4$ 18 $pi21$ 4Owarihatamochi19 $Pi22(i)$ 6Suweon36520 $Pi23$ 5Suweon36521 $Pi25$ 6Gumei 223 $Pi25(i)$ 2 $IR6$ 24 $Pi26$ 6Gumei 225 $Pi26(i)$ 5 $Azucena$ 26 $Pi27$ 1 $Q14$ 27 $Pi27(i)$ 6 $IR64$ 38 $Pi30(i)$ 11 $IR64$ 39 $Pi32(i)$ 12 $IR64$ 34 $Pi33$ 8 $IR64$ 35 $Pi34$ 11 $Chubu32$ 36 $Pi35(i)$ 1 $Hokai 188$ 37 $Pi38$ 11 $Tadukan$				
7 $Pil1$ 8 $Zhai-Ya-Quing8$ 8 $Pil2$ 12K80-R-Hang, Jiao-Zhan, Moroberekan9 $Pil3(t)$ 6 $O.$ minuta (W), Kasalath (I),Maowangu10 $Pil4(t)$ 2Maowangu11 $Pil5$ 9GA2512 $Pil5(t)$ 12Moroberekan13 $Pil6(t)$ 2Aus37314 $Pil7$ 7DJ12315 $Pil8(t)$ 11Suweon36516 $Pil9(t)$ 12Aichi Asahi17 $Pi20$ 12IR2418 $pi21$ 4Owarihatamochi19 $Pi22(t)$ 6Suweon36520 $Pi23$ 5Suweon36521 $Pi24(t)$ 1Azucena22 $Pi25$ 6Gumei 223 $Pi25(t)$ 2IR624 $Pi26$ 6Gumei 225 $Pi26(t)$ 5Azucena26 $Pi27$ 1Q1427 $Pi20(t)$ 8IR6430 $Pi3(t)$ 10IR6431 $Pi30(t)$ 11IR6432 $Pi31(t)$ 12IR6433 $Pi32(t)$ 12IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(t)$ 1Hokkai 18837 $Pi38$ 11Tadukan				
8 $Pil2$ 12K80-R-Hang, Jiao-Zhan, Moroberekan9 $Pil3(t)$ 6 $O.$ minuta (W), Kasalath (I), Maowangu10 $Pil4(t)$ 2Maowangu11 $Pil5$ 9GA2512 $Pil5(t)$ 12Moroberekan13 $Pil6(t)$ 2Aus37314 $Pil7$ 7DJ12315 $Pil8(t)$ 11Suweon36516 $Pil9(t)$ 12Aichi Asahi17 $Pi20$ 12IR2418 $pi21$ 4Owarihatamochi19 $Pi22(t)$ 6Suweon36520 $Pi23$ 5Suweon36521 $Pi24(t)$ 1Azucena22 $Pi25$ 6Gumei 223 $Pi25(t)$ 2IR624 $Pi26$ 6Gumei 225 $Pi26(t)$ 5Azucena26 $Pi27$ 1Q1427 $Pi27(t)$ 6IR6428 $Pi28(t)$ 10IR6429 $Pi29(t)$ 8IR6430 $Pi3(t)$ 6Pai-kan-tao31 $Pi30(t)$ 11IR6432 $Pi31(t)$ 12IR6433 $Pi32(t)$ 12IR6434 $Pi33$ 8IR6435 $Pi35(t)$ 1Hokkai 18837 $Pi36$ 8Q6138 $Pi37$ 1St- No 139 $Pi38$ 11Tadukan				-
9 $Pi13(t)$ 6 $O.\ minuta$ (W), Kasalath (I), Maowangu10 $Pi14(t)$ 2Maowangu11 $Pi15$ 9GA2512 $Pi15(t)$ 12Moroberekan13 $Pi16(t)$ 2Aus37314 $Pi17$ 7DJ12315 $Pi18(t)$ 11Suweon36516 $Pi19(t)$ 12Aichi Asahi17 $Pi20$ 12IR2418 $pi21$ 4Ovarihatamochi19 $Pi22(t)$ 6Suweon36520 $Pi23$ 5Suweon36521 $Pi24(t)$ 1Azucena22 $Pi25$ 6Gumei 223 $Pi25(t)$ 2IR624 $Pi26$ 6Gumei 225 $Pi27$ 1Q1427 $Pi27(t)$ 6IR6428 $Pi28(t)$ 10IR6429 $Pi29(t)$ 8IR6430 $Pi3(t)$ 6Pai-kan-tao31 $Pi30(t)$ 11IR6432 $Pi31$ 8IR6433 $Pi32(t)$ 12IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(t)$ 1Hokkai 18837 $Pi36$ 8Q6138 $Pi38$ 11Tadukan				
10 $PiI4(t)$ 2Maowangu11 $PiI5$ 9GA2512 $PiI5(t)$ 12Moroberekan13 $PiI6(t)$ 2Aus37314 $PiI7$ 7DJ12315 $Pi18(t)$ 11Suweon36516 $Pi19(t)$ 12Aichi Asahi17 $Pi20$ 12IR2418 $pi21$ 4Owarihatamochi19 $Pi22(t)$ 6Suweon36520 $Pi23$ 5Suweon36521 $Pi24(t)$ 1Azucena22 $Pi25$ 6Gumei 223 $Pi25(t)$ 2IR624 $Pi26$ 6Gumei 225 $Pi26(t)$ 5Azucena26 $Pi27$ 1Q1427 $Pi27(t)$ 6IR6428 $Pi28(t)$ 10IR6430 $Pi3(t)$ 6Pai-kan-tao31 $Pi30(t)$ 11IR6432 $Pi31(t)$ 12IR6433 $Pi32(t)$ 12IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(t)$ 1Hokkai 18837 $Pi36$ 8Q6138 $Pi38$ 11Tadukan				
11 $Pi15$ 9GA2512 $Pi15(t)$ 12Moroberekan13 $Pi16(t)$ 2Aus37314 $Pi17$ 7DJ12315 $Pi18(t)$ 11Suweon36516 $Pi19(t)$ 12Aichi Asahi17 $Pi20$ 12IR2418 $pi21$ 4Owarihatamochi19 $Pi22(t)$ 6Suweon36520 $Pi23$ 5Suweon36521 $Pi24(t)$ 1Azucena22 $Pi25$ 6Gumei 223 $Pi25(t)$ 2IR624 $Pi26$ 6Gumei 225 $Pi26(t)$ 5Azucena26 $Pi27$ 1Q1427 $Pi27(t)$ 6IR6428 $Pi28(t)$ 10IR6430 $Pi3(t)$ 6Pai-kan-tao31 $Pi30(t)$ 11IR6432 $Pi31(t)$ 12IR6433 $Pi33$ 8IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(t)$ 1Hokkai 18837 $Pi36$ 8Q6138 $Pi38$ 11Tadukan				
12 $Pi15(t)$ 12Moroberekan13 $Pi16(t)$ 2Aus37314 $Pi17$ 7DJ12315 $Pi18(t)$ 11Suweon36516 $Pi19(t)$ 12Aichi Asahi17 $Pi20$ 12IR2418 $pi21$ 4Owarihatamochi19 $Pi22(t)$ 6Suweon36520 $Pi23$ 5Suweon36521 $Pi24(t)$ 1Azucena22 $Pi25$ 6Gumei 223 $Pi25(t)$ 2IR624 $Pi26$ 6Gumei 225 $Pi26(t)$ 5Azucena26 $Pi27$ 1Q1427 $Pi27(t)$ 6IR6428 $Pi28(t)$ 10IR6430 $Pi3(t)$ 6Pai-kan-tao31 $Pi30(t)$ 11IR6433 $Pi32(t)$ 12IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(t)$ 1Hokkai 18837 $Pi36$ 8Q6138 $Pi37$ 1St-No 139 $Pi38$ 11Tadukan				-
13 $Pi16(i)$ 2Aus37314 $Pi17$ 7DJ12315 $Pi18(i)$ 11Suweon36516 $Pi19(i)$ 12Aichi Asahi17 $Pi20$ 12IR2418 $pi21$ 4Owarihatamochi19 $Pi22(i)$ 6Suweon36520 $Pi23$ 5Suweon36521 $Pi24(i)$ 1Azucena22 $Pi25$ 6Gumei 223 $Pi25(i)$ 2IR624 $Pi26$ 6Gumei 225 $Pi26(i)$ 5Azucena26 $Pi27$ 1Q1427 $Pi27(i)$ 6IR6428 $Pi28(i)$ 10IR6429 $Pi29(i)$ 8IR6430 $Pi3(i)$ 6Pai-kan-tao31 $Pi30(i)$ 11IR6433 $Pi32(i)$ 12IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(i)$ 1Hokkai 18837 $Pi36$ 8Q6138 $Pi37$ 1St-No 139 $Pi38$ 11Tadukan				
14 $Pi17$ 7DJ12315 $Pi18(t)$ 11Suweon36516 $Pi19(t)$ 12Aichi Asahi17 $Pi20$ 12IR2418 $pi21$ 4Owarihatamochi19 $Pi22(t)$ 6Suweon36520 $Pi23$ 5Suweon36521 $Pi24(t)$ 1Azucena22 $Pi25$ 6Gumei 223 $Pi25(t)$ 2IR624 $Pi26$ 6Gumei 225 $Pi26(t)$ 5Azucena26 $Pi27$ 1Q1427 $Pi27(t)$ 6IR6428 $Pi28(t)$ 10IR6430 $Pi3(t)$ 6Pai-kan-tao31 $Pi30(t)$ 11IR6432 $Pi31(t)$ 12IR6433 $Pi32(t)$ 12IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(t)$ 1Hokkai 18837 $Pi36$ 8Q6138 $Pi37$ 1St- No 139 $Pi38$ 11Tadukan				
15 $Pi18(t)$ 11Suweon36516 $Pi19(t)$ 12Aichi Asahi17 $Pi20$ 12IR2418 $pi21$ 4Owarihatamochi19 $Pi22(t)$ 6Suweon36520 $Pi23$ 5Suweon36521 $Pi24(t)$ 1Azucena22 $Pi25$ 6Gumei 223 $Pi25(t)$ 2IR624 $Pi26$ 6Gumei 225 $Pi26(t)$ 5Azucena26 $Pi27$ 1Q1427 $Pi27(t)$ 6IR6428 $Pi28(t)$ 10IR6430 $Pi3(t)$ 6Pai-kan-tao31 $Pi30(t)$ 11IR6432 $Pi31(t)$ 12IR6433 $Pi32(t)$ 12IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(t)$ 1Hokkai 18837 $Pi36$ 8Q6138 $Pi37$ 1St- No 139 $Pi38$ 11Tadukan				
16 $Pi19(t)$ 12Aichi Asahi17 $Pi20$ 12IR2418 $pi21$ 4Owarihatamochi19 $Pi22(t)$ 6Suweon36520 $Pi23$ 5Suweon36521 $Pi24(t)$ 1Azucena22 $Pi25$ 6Gumei 223 $Pi25(t)$ 2IR624 $Pi26$ 6Gumei 225 $Pi26(t)$ 5Azucena26 $Pi27$ 1Q1427 $Pi27(t)$ 6IR6428 $Pi28(t)$ 10IR6429 $Pi29(t)$ 8IR6430 $Pi3(t)$ 6Pai-kan-tao31 $Pi30(t)$ 11IR6432 $Pi31(t)$ 12IR6433 $Pi32(t)$ 12IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(t)$ 1Hokkai 18837 $Pi36$ 8Q6138 $Pi37$ 1St-No 139 $Pi38$ 11Tadukan				
17 $Pi20$ $12$ $IR24$ $18$ $pi21$ 4Owarihatamochi $19$ $Pi22(t)$ 6Suweon365 $20$ $Pi23$ 5Suweon365 $21$ $Pi24(t)$ 1Azucena $22$ $Pi25$ 6Gumei 2 $23$ $Pi25(t)$ 2IR6 $24$ $Pi26$ 6Gumei 2 $25$ $Pi26(t)$ 5Azucena $26$ $Pi27$ 1Q14 $27$ $Pi27(t)$ 6IR64 $28$ $Pi28(t)$ 10IR64 $29$ $Pi29(t)$ 8IR64 $30$ $Pi3(t)$ 6Pai-kan-tao $31$ $Pi30(t)$ 11IR64 $32$ $Pi31(t)$ 12IR64 $34$ $Pi33$ 8IR64 $35$ $Pi34$ 11Chubu32 $36$ $Pi35(t)$ 1Hokkai 188 $37$ $Pi36$ 8Q61 $38$ $Pi37$ 1St- No 1 $39$ $Pi38$ 11Tadukan				
18 $pi21$ 4Owarihatamochi19 $Pi22(t)$ 6Suweon36520 $Pi23$ 5Suweon36521 $Pi24(t)$ 1Azucena22 $Pi25$ 6Gumei 223 $Pi25(t)$ 2IR624 $Pi26$ 6Gumei 225 $Pi26(t)$ 5Azucena26 $Pi27$ 1Q1427 $Pi27(t)$ 6IR6428 $Pi28(t)$ 10IR6429 $Pi29(t)$ 8IR6430 $Pi3(t)$ 6Pai-kan-tao31 $Pi30(t)$ 11IR6432 $Pi31(t)$ 12IR6433 $Pi32(t)$ 12IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(t)$ 1Hokkai 18837 $Pi36$ 8Q6138 $Pi37$ 1St- No 139 $Pi38$ 11Tadukan	16	<i>Pi19(t)</i>		
19 $Pi22(t)$ 6Suweon36520 $Pi23$ 5Suweon36521 $Pi24(t)$ 1Azucena22 $Pi25$ 6Gumei 223 $Pi25(t)$ 2IR624 $Pi26$ 6Gumei 225 $Pi26(t)$ 5Azucena26 $Pi27$ 1Q1427 $Pi27(t)$ 6IR6428 $Pi28(t)$ 10IR6429 $Pi29(t)$ 8IR6430 $Pi3(t)$ 6Pai-kan-tao31 $Pi30(t)$ 11IR6432 $Pi31(t)$ 12IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(t)$ 1Hokkai 18837 $Pi36$ 8Q6138 $Pi37$ 1St- No 139 $Pi38$ 11Tadukan		Pi20		
20 $Pi23$ 5Suweon365 $21$ $Pi24(t)$ 1Azucena $22$ $Pi25$ 6Gumei 2 $23$ $Pi25(t)$ 2IR6 $24$ $Pi26$ 6Gumei 2 $25$ $Pi26(t)$ 5Azucena $26$ $Pi27$ 1Q14 $27$ $Pi27(t)$ 6IR64 $28$ $Pi28(t)$ 10IR64 $29$ $Pi29(t)$ 8IR64 $30$ $Pi3(t)$ 6Pai-kan-tao $31$ $Pi30(t)$ 11IR64 $32$ $Pi31(t)$ 12IR64 $33$ $Pi32(t)$ 12IR64 $34$ $Pi33$ 8IR64 $35$ $Pi34$ 11Chubu32 $36$ $Pi35(t)$ 1Hokkai 188 $37$ $Pi36$ 8Q61 $38$ $Pi37$ 1St- No 1 $39$ $Pi38$ 11Tadukan		*		Owarihatamochi
21 $Pi24(i)$ 1Azucena $22$ $Pi25$ 6Gumei 2 $23$ $Pi25(i)$ 2IR6 $24$ $Pi26$ 6Gumei 2 $25$ $Pi26(i)$ 5Azucena $26$ $Pi27$ 1Q14 $27$ $Pi27(i)$ 6IR64 $28$ $Pi28(i)$ 10IR64 $29$ $Pi29(i)$ 8IR64 $30$ $Pi3(i)$ 6Pai-kan-tao $31$ $Pi30(i)$ 11IR64 $32$ $Pi31(i)$ 12IR64 $33$ $Pi32(i)$ 12IR64 $34$ $Pi33$ 8IR64 $35$ $Pi34$ 11Chubu32 $36$ $Pi35(i)$ 1Hokkai 188 $37$ $Pi36$ 8Q61 $38$ $Pi37$ 1St- No 1 $39$ $Pi38$ 11Tadukan	19			Suweon365
22 $Pi25$ 6Gumei 2 $23$ $Pi25(t)$ 2IR6 $24$ $Pi26$ 6Gumei 2 $25$ $Pi26(t)$ 5Azucena $26$ $Pi27$ 1Q14 $27$ $Pi27(t)$ 6IR64 $28$ $Pi28(t)$ 10IR64 $29$ $Pi29(t)$ 8IR64 $30$ $Pi3(t)$ 6Pai-kan-tao $31$ $Pi30(t)$ 11IR64 $32$ $Pi31(t)$ 12IR64 $34$ $Pi33$ 8IR64 $35$ $Pi34$ 11Chubu32 $36$ $Pi35(t)$ 1Hokkai 188 $37$ $Pi36$ 8Q61 $38$ $Pi37$ 1St-No 1 $39$ $Pi38$ 11Tadukan				Suweon365
23 $Pi25(t)$ 2IR624 $Pi26$ 6Gumei 225 $Pi26(t)$ 5Azucena26 $Pi27$ 1Q1427 $Pi27(t)$ 6IR6428 $Pi28(t)$ 10IR6429 $Pi29(t)$ 8IR6430 $Pi3(t)$ 6Pai-kan-tao31 $Pi30(t)$ 11IR6432 $Pi31(t)$ 12IR6433 $Pi32(t)$ 12IR6434 $Pi33$ 8IR6435 $Pi34$ 11Chubu3236 $Pi35(t)$ 1Hokkai 18837 $Pi36$ 8Q6138 $Pi37$ 1St- No 139 $Pi38$ 11Tadukan		Pi24(t)		Azucena
24 $Pi26$ 6Gumei 2 $25$ $Pi26(t)$ 5Azucena $26$ $Pi27$ 1 $Q14$ $27$ $Pi27(t)$ 6 $IR64$ $28$ $Pi28(t)$ 10 $IR64$ $29$ $Pi29(t)$ 8 $IR64$ $30$ $Pi3(t)$ 6 $Pai$ -kan-tao $31$ $Pi30(t)$ 11 $IR64$ $32$ $Pi31(t)$ 12 $IR64$ $33$ $Pi32(t)$ 12 $IR64$ $34$ $Pi33$ 8 $IR64$ $35$ $Pi34$ 11 $Chubu32$ $36$ $Pi35(t)$ 1Hokkai 188 $37$ $Pi36$ 8 $Q61$ $38$ $Pi37$ 1 $St$ -No 1 $39$ $Pi38$ 11Tadukan				
25 $Pi26(t)$ 5Azucena26 $Pi27$ 1 $Q14$ 27 $Pi27(t)$ 6 $IR64$ 28 $Pi28(t)$ 10 $IR64$ 29 $Pi29(t)$ 8 $IR64$ 30 $Pi3(t)$ 6 $Pai$ -kan-tao31 $Pi30(t)$ 11 $IR64$ 32 $Pi31(t)$ 12 $IR64$ 33 $Pi32(t)$ 12 $IR64$ 34 $Pi33$ 8 $IR64$ 35 $Pi34$ 11 $Chubu32$ 36 $Pi35(t)$ 1 $Hokkai 188$ 37 $Pi36$ 8 $Q61$ 38 $Pi37$ 1 $St$ - No 139 $Pi38$ 11Tadukan				
26 $Pi27$ 1 $Q14$ $27$ $Pi27(t)$ 6IR64 $28$ $Pi28(t)$ 10IR64 $29$ $Pi29(t)$ 8IR64 $30$ $Pi3(t)$ 6Pai-kan-tao $31$ $Pi30(t)$ 11IR64 $32$ $Pi31(t)$ 12IR64 $33$ $Pi32(t)$ 12IR64 $34$ $Pi33$ 8IR64 $35$ $Pi34$ 11Chubu32 $36$ $Pi35(t)$ 1Hokkai 188 $37$ $Pi36$ 8Q61 $38$ $Pi37$ 1St-No 1 $39$ $Pi38$ 11Tadukan				Gumei 2
27 $Pi27(t)$ 6IR64 $28$ $Pi28(t)$ 10IR64 $29$ $Pi29(t)$ 8IR64 $30$ $Pi3(t)$ 6Pai-kan-tao $31$ $Pi30(t)$ 11IR64 $32$ $Pi31(t)$ 12IR64 $33$ $Pi32(t)$ 12IR64 $34$ $Pi33$ 8IR64 $35$ $Pi34$ 11Chubu32 $36$ $Pi35(t)$ 1Hokkai 188 $37$ $Pi36$ 8Q61 $38$ $Pi37$ 1St- No 1 $39$ $Pi38$ 11Tadukan		Pi26(t)		
28 $Pi28(t)$ 10IR64 $29$ $Pi29(t)$ 8IR64 $30$ $Pi3(t)$ 6Pai-kan-tao $31$ $Pi30(t)$ 11IR64 $32$ $Pi31(t)$ 12IR64 $33$ $Pi32(t)$ 12IR64 $34$ $Pi33$ 8IR64 $35$ $Pi34$ 11Chubu32 $36$ $Pi35(t)$ 1Hokkai 188 $37$ $Pi36$ 8Q61 $38$ $Pi37$ 1St- No 1 $39$ $Pi38$ 11Tadukan				
29 $Pi29(t)$ 8IR64 $30$ $Pi3(t)$ 6Pai-kan-tao $31$ $Pi30(t)$ 11IR64 $32$ $Pi31(t)$ 12IR64 $33$ $Pi32(t)$ 12IR64 $34$ $Pi33$ 8IR64 $35$ $Pi34$ 11Chubu32 $36$ $Pi35(t)$ 1Hokkai 188 $37$ $Pi36$ 8Q61 $38$ $Pi37$ 1St- No 1 $39$ $Pi38$ 11Tadukan				IR64
30       Pi3(t)       6       Pai-kan-tao         31       Pi30(t)       11       IR64         32       Pi31(t)       12       IR64         33       Pi32(t)       12       IR64         34       Pi33       8       IR64         35       Pi34       11       Chubu32         36       Pi35(t)       1       Hokkai 188         37       Pi36       8       Q61         38       Pi37       1       St- No 1         39       Pi38       11       Tadukan			10	IR64
31       Pi30(t)       11       IR64         32       Pi31(t)       12       IR64         33       Pi32(t)       12       IR64         34       Pi33       8       IR64         35       Pi34       11       Chubu32         36       Pi35(t)       1       Hokkai 188         37       Pi36       8       Q61         38       Pi37       1       St- No 1         39       Pi38       11       Tadukan	29		8	IR64
32       Pi31(t)       12       IR64         33       Pi32(t)       12       IR64         34       Pi33       8       IR64         35       Pi34       11       Chubu32         36       Pi35(t)       1       Hokkai 188         37       Pi36       8       Q61         38       Pi37       1       St- No 1         39       Pi38       11       Tadukan		Pi3(t)		
33       Pi32(t)       12       IR64         34       Pi33       8       IR64         35       Pi34       11       Chubu32         36       Pi35(t)       1       Hokkai 188         37       Pi36       8       Q61         38       Pi37       1       St- No 1         39       Pi38       11       Tadukan				
34         Pi33         8         IR64           35         Pi34         11         Chubu32           36         Pi35(t)         1         Hokkai 188           37         Pi36         8         Q61           38         Pi37         1         St- No 1           39         Pi38         11         Tadukan				
35       Pi34       11       Chubu32         36       Pi35(t)       1       Hokkai 188         37       Pi36       8       Q61         38       Pi37       1       St- No 1         39       Pi38       11       Tadukan				
36       Pi35(t)       1       Hokkai 188         37       Pi36       8       Q61         38       Pi37       1       St- No 1         39       Pi38       11       Tadukan	34	Pi33	8	IR64
37     Pi36     8     Q61       38     Pi37     1     St- No 1       39     Pi38     11     Tadukan				
38         Pi37         1         St- No 1           39         Pi38         11         Tadukan	36	Pi35(t)		
39 <i>Pi38</i> 11 Tadukan				Q61
		Pi37		St- No 1
40 <i>Pi39(t)</i> 4,12 Chubu 111, Q15	39	Pi38		
	40	Pi39(t)	4,12	Chubu 111, Q15

## Table 1. Blast resistance genes reported in rice.

Contd....



Sl. No.	Gene name	Location (Chr No)	Sources of resistance
41	Pi40(t)	6	O. australiensis
42	Pi41	12	93-11
43	Pi42(t)	12	DHR9
44	Pi44	11	Moroberekan
45	<i>Pi47</i>	11	Xiangzi 3150
46	Pi48	12	Xiangzi 3150
47	Pi5(t)	9	Moroberekan
48	Pi6(t)	12	Apura
49	Pi62(t)	12	Yashiro-mochi
50	Pi67		Tsuyuake
51	Pi8	6	Kasalath
52	Pi9	6	O. minuta
53	Pia	11	Aichi Asahi
54	Pib	2	Tohoku IL9
55	Pib2	11	Lemont
56	PiCO39(t)	11	CO39
57	Pid(t)1	2	Digu
58	Pid2	6	Digu
59	Pif	11	Chugoku 31-1
60	Pig(t)	2	Guangchangzhan
61	PiGD1	8	Sanhuangzhan 2
62	PiGD-2	10	Sanhuangzhan 2
63	PiGD3	12	Sanhuangzhan 2
64	Pigm(t)	6	Gumei4
65	Pii	9	Ishikari Shiroke, Fujisaa5
66	Piil	6	Fujisaka 5
67	Pii2	9	Ishikari Shiroke
68	Piis1	11	ImochiShirazu
69	Piis2	-	ImochiShirazu
70	Piis3	-	ImochiShirazu
71	Pik	11	Kusabue
72	Pikg	11	GA20
73	Pikh (Pi54)	11	Tetep
74	Pikm	11	Tsuyuake
75	Pikp	11	HR22
76	Piks	11	Shin 2
77	Pikur1	4	Kuroka
78	Pikur2	11	Kuroka
79	Pilm2	11	Lemont
80	<i>Pir2-3(t)</i>	2	IR64
81	Pirf2-1(t)	2	O. rufipogon

Contd....



Sl. No.	Gene name	Location (Chr No)	Sources of resistance
82	Pise	11	Sensho
83	Pise2	-	Sensho
84	Pise3	-	Sensho
85	Pish	1	Shin 2
86	Pish	11	Nipponbare
87	Pit	1	Tjahaja
88	Pita	12	Tadukan
89	Pita2	12	Shimokita
90	Pitp(t)	1	Tetep
91	Pitq1	6	Teqing
92	Pitq2	2	Teqing
93	Pitq3	3	Teqing
94	Pitq4	4	Teqing
95	Pi-tq5	2	Teqing
96	Pitq6	12	Teqing
97	Piyl(t)	2	Yanxian No 1
98	Piy2(t)	2	Yanxian No 1
99	Piz	6	Zenith (J), Fukunishiki, Toride 1, Tadukan
100	Pizh	8	Zhai-Ya-Quing8
101	Pi157	12	Moroberekan
102	Pi-jnw1	11	Jiangnanwan

Adapted and updated from Sharma et al. (2012)

susceptible cultivar CO39. It was reported that plants carrying two or three gene combinations showed enhanced resistance as compared to *Piz-5* alone. Singh et al. (2011) improved the parental lines of rice hybrid Pusa RH 10 by introgressing the blast resistant gene *Pi 54* into them. The group has also developed and released a blast-resistant basmati variety, Pusa Basmati 1637 through transfer of *Pi9* using marker-assisted selection. Introgression of blast resistance genes *Pi1*, *Pi2* and *Pi33* into rice variety ADT43 was carried out at Tamil Nadu Agricultural University, Coimbatore.

At The National Rice Research Institute, Yadav et al. (2017) attempted to find out the status of twelve major blast resistance genes and their diversity among eighty released rice varieties of the institute (National Rice Research Institute, Cuttack). Linked molecular markers for genes *Pib*, *Piz*, *Piz-t*, *Pik*, *Pik-p*, *PikmPik-h*, *Pita/Pita-*2, *Pi2*, *Pi9*, *Pi1* and *Pi5* were used in the study. Among the 80 varieties used, 19 were resistant, 21 were moderately resistant and 40 were susceptible to the disease. The blast resistance genes in the different varieties varied from 4 to 12 and the frequencies of the resistance genes ranged from 0 to100%.

Marker assisted backcross breeding strategy was applied for pyramiding blast resistance genes (*Pi2* and *Pi9*), into Vandana and Kalinga III through the crosses (Kalinga III/C101A51 (*Pi-2(t)*)//KalingaIII/O. *minute* der. WHD IS 75-127(*Pi-9(t)*) and Vandana/C101A51//Vandana/O. *minute* der. WHD IS 75-127). Many lines in the



background of Vandana and Kalinga III were developed. Among the promising lines, CR 2619-2, CR 2619-5, CR 2619-6, CR 2619-7, CR 2619-8 and CR 2619-9 are in the background of Vandana while CR 2620-1, CR 2620-2, CR 2620-3 and CR 2620-4 are in Kalinga III background. The promising lines were tested in Disease Screening Nursery (DSN) under AICRIP for multi-location trials.

#### 2.1. Bacterial blight (Xanthomonas oryzae pv. oryzae) resistance

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is a devastating disease in the rice-growing countries of Asia. Infection at maximum tillering stage results in blighting of leaves, which eventually causes significant yield losses in severely infected fields ranging from 20 to 30%, but this, can reach as high as 80%. Development of cultivars carrying major resistance (R) genes have been the most effective and economic strategy to control BB disease. To date, at least 38 BB resistance genes conferring host resistance against various strains of *Xoo* have been identified (Table 2). All of these genes follow a Mendelian pattern of inheritance and express resistance to a diverse group of *Xoo* pathogens. Several of these genes have already been incorporated into rice cultivars, which are now widely cultivated in many countries. BB resistance genes *Xa 33(t)* and *Xa 38* were identified from *Oryza nivara*. A new mutant named 'XM14' obtained from IR24, which was found to be resistant to all Japanese *Xoo* races. The gene identified in XM14 was designated as *xa42*.

In IRRI, IR24 NILs (IRBB lines) containing *Xa4*, *xa5*, *xa13* and *Xa21* genes and their combinations were developed which were extensively used in the breeding programmes of many countries including India. Indian scientists from the National Agricultural Research and Education System used these IRBB lines for transfer of BB resistance genes in many popular high yielding varieties. The gene combinations chosen by breeders, however, remained confined to *xa13* and *Xa21* or *xa5*, *xa13* and *Xa21*. However, Ellur et al. (2016) incorporated *Xa38* in the basmati background of PB1121 and found that it provides resistance to an additional race of the pathogen when compared with its NIL pyramided with *xa13+Xa21*.

The *Xa21* gene was identified at NRRI in the wild species *Oryza longistaminata*, which was highly effective against BB races in South and Southeastern Asia. The gene was later mapped and cloned at IRRI and is being extensively utilized by breeders across the globe. Varietal improvement programme was initiated to improve the BB resistance in popular high yielding varieties as recurrent parents and BB resistance genotypes viz., Ajaya (*xa5*), IRBB 8 (*xa8*) and IRBB 60 (*xa5*, *xa13 and Xa21*) as donors through backcross breeding coupled with artificial screening.

Resistance genes (*xa5*, *xa13* and *Xa21*; either singly or in different combinations) pyramided lines were developed through marker assisted backcross breeding in the genetic background of Swarna and IR64 under the Asian Rice Biotechnology Network (Reddy et. al. 1997). The promising pyramided lines identified through DSN of AICRIP in different locations across the country were recommended for registration for their



Xa gene	Resistance to Xoo race	Donor cultivar	Chr.
Xal	Japanese race -I	Kogyoku, IRBB 1	4
Xa2	Japanese race -II	IRBB2	4
Xa3/Xa26	Chinese, Philippine, and	Wase Aikoku 3, Minghui 63, IRBB3	11
Xa4	Japanese races Philippine race-I	TKM6, IRBB4	11
ли4 ха5	Philippine races-I, II, III	IRBB5	5
хаб Хаб	Philippine race-I	Zenith	11
Xa0 Xa7	Philippine races	DZ78	6
ли/ xa8	Philippine races	P1231128	7
xa9	Philippine races	Khao Lay Nhay and Sateng	11
ха9 Ха10	11	Cas 209	11
Xall	Philippine and Japanese races	IRS	3
	Japanese races IB, II, IIIA, V		
Xa12	Indonesian race-V	Kogyoku, Java14	4
xal3 Val4	Philippine race 6	BJ1, IRBB13	8
Xal4	Philippine race 5	TN1	4
xa15	Japanese races	M41 Mutant	-
Xa16	Japanese races	Tetep	-
Xa17	Japanese races	Asominori	-
Xa18	Burmese races	IR24, Miayang 23, Toyonishiki	-
xa19	Japanese races	XM5 (Mutant of IR24)	-
xa20	Japanese races	XM6 (Mutant of IR24)	-
Xa21	Philippine and Japanese races	O. longistaminata, IRBB21	11
Xa22	Chinese races	Zhachanglong	11
Xa23	Indonesian races	O. rufipogon (CBB23)	11
xa24(t)	Philippine and Chinese races	DV86	2
Xa25	Chinese and Philippine races	Minghui 63, HX-3 (Somoclonal mutant of Minghui 63)	12
xa26(t)	Philippine races	Nep Bha Bong	-
Xa27	Chinese strains and Philippine race 2 to 6	O. minuta, IRGC 101141, IRBB27	6
xa28 (t)	Philippine race 2	Lota sail	-
Xa29(t)	Chinese races	<i>O. officinalis</i> (B5)	1
Xa30 (t)	Indonesian races	O.rufipogon (Y235)	11
Xa31(t)	Chinese races	Zhachanglong	4
Xa32(t)	Philippine races	<i>O. australiensis</i> (introgression line C4064)	11
xa33(t)	Thai races	Ba7 <i>O. nivara</i>	6
Xa33(t)			
Xa34 (t)	Thai races	BG1222	1
Xa35(t)	Philippine races	<i>O. minuta</i> (Acc. No.101133)	11
Xa36(t)	Philippine races	C4059	11
Xa38	Indian Punjab races	O. nivara IRGC81825	4
Xa39	Chinese and Philippine races	FF329	11
Xa40(t)	Korean BB races	IR65482-7-216-1-2	11
xa41(t)	Various Xoo strains	Rice germplasm	-
xa42	Japanese Xoo races	XM14, a mutant of IR24	3
xu42		ANTIT, a mutant of m27	5

## Table 2. List of BB resistance genes reported in rice.

Adapted and updated from Kou and Wang (2013).



use as potential donors in future breeding programmes (DRR Annual Progress Report 2003; 2005). Two lines CRMAS 2231-37 (IET 20668) and CRMAS 2231-48 (IET 20669) in the background of IR 64 were found promising for BB endemic areas of Uttarakhand and Andhra Pradesh and Uttarakhand and Haryana, respectively while one line CRMAS 2232-85 (ET 20672) in the background of Swarna was recommended for the endemic areas of Gujrat and Maharashtra. Pradhan et al. (2015) introgressed three BB resistance genes (xa5, xa13 and Xa21) by marker-assisted backcrossing, in the background of the popular, but highly BB susceptible deepwater variety, Jalmagna. The pyramided lines showed a high level of BB resistance and significant yield advantage over Jalmagna under conditions of BB infection. Lines carrying two BB gene combinations (Xa21+xa13 and Xa21+xa5) were also developed in the background of Jalmagna (Pradhan et al. 2016). The pyramided lines showed increased resistance to BB isolates prevalent in the region. The parental line improvement for BB resistance has been successfully undertaken at NRRI in case of popular rice hybrid Rajalaxmi, by introgressing four resistance genes (Xa4, xa5, xa13, and Xa21) through Markerassisted backcross (MAB) breeding (Dash et al. 2016).

Varietal improvement program at NRRI for BB resistance resulted in the release of Improved Lalat [CRMAS 2621-7-1 (IET 21066)], Improved Tapaswini [CRMAS 2622-7-6 (IET 21070)] and CR Dhan 800 in the genetic background of popular rice varieties Lalat, Tapaswini and Swarna, respectively. Improved Lalat and Improved Tapaswini carry four genes (*Xa4, xa5, xa13* and *Xa21*) while CR Dhan 800 has three resistance genes *Xa21, xa13* and *xa5*. All have been effective for growing in the "bacterial blight" endemic areas of Odisha.

# 3. SHEATH BLIGHT (*RHIZOCTONIA SOLANI* KUHN) TOLERANCE/RESISTANCE

Sheath blight of rice, caused by the fungus, *Rhizoctonia solani* Kuhn, is becoming a major threat to rice production worldwide. Though first reported as early as in 1910, sheath blight became a prominent disease only after the introduction of high yielding semi-dwarf varieties in the 1960's. The intensive cropping involving cultivation of a single variety over a large area and the high use of nitrogenous fertilizer led to a dramatic increase in the incidence of sheath blight in major rice-growing countries of the world as well as India. Almost all the prominent varieties grown in the country are highly susceptible to the disease. Development of genotypes tolerant to the disease is considered as the most sustainable, eco-friendly and economic way to combat the disease.

Breeding for sheath blight (ShB) tolerance in rice poses many unique challenges compared to other pests and diseases. Being caused by a necrotrophic fungus, ShB tolerance is a quantitative trait governed by polygenes. Lack of a well-standardized screening protocol compounded with the influence of environment and various plant morphological features on trait expression make identification of truly resistant lines a daunting task. Genotypes with moderate disease resistance have been reported in the past, but a strong ShB resistant source is yet to be identified from both the cultivated and wild gene pool of rice.

59



From the moderate resistance sources identified, more than hundred QTLs (Table 3) have been reported for ShB tolerance in rice, but most of them have minor effects and are correlated with various plant morphological features, especially plant height and heading date. Even for the major ShB QTLs having plant morphology-independent effect, the expression is highly affected by the genetic background, limiting the usefulness of the QTLs in practical plant breeding. The breeding potential of few ShB QTLs viz.,  $qSB9-2^{TQ}$ ,  $qSB-11^{LE}$  and  $qSB-9^{TQ}$  have been tested in different genetic backgrounds and their effect on sheath blight tolerance was validated. Two of these QTLs,  $qSB-11^{LE}$  and qSB-9TQ were fine mapped.

There are only limited reports of utilization of identified ShB QTLs in practical plant breeding, with only limited resistance genotypes viz., Teging, Tetep, Lemont and Jasmine 85 being regularly used as donors of ShB tolerance. Pinson et al. (2008) have improved the ShB tolerance of the popular American rice genotype Lemont by introgressing ShB tolerance QTLs from TeQing. Three TeQing-into-Lemont backcross introgression lines (TILs) containing eight ShB QTLs and having significantly less sheath blight susceptibility compared to the recurrent parent were released in the USA in 2007. Wang et al. (2012) have developed TeQing-into-Lemont backcross introgression lines (TILs) of QTLs qSB9-2 and qSB12-1 and found that resistant alleles of the QTLs from TeQing significantly improved ShB tolerance of the TILs. Chen et al. (2014) have transferred the QTLs qSB-7 and qSB-9 from Teqing into the genetic background of commercial japonica varieties by MAS. The two QTLs were also pyramided in the background of the japonica variety WLJ1. There was a significant reduction in SB incidence and yield loss in the introgressed lines and pyramiding of two QTLs were found to be more effective rather than using single QTL. Zuo et al. (2014) have shown that pyramiding of QTLs for ShB tolerance and tiller angle, qSB- $9^{TQ}$  and  $TACI^{TQ}$ , had significantly increased disease tolerance in the near-isogenic lines (NILs) carrying them. Both the QTLs have improved the ShB tolerance of the NILs but qSB-9<sup>TQ</sup> was more effective than TAC1<sup>TQ</sup>. The NILs having both the QTLs had more tolerance to sheath blight compared to the NILs having any one of them.

In India, ShB tolerance breeding relies mainly on the genotype Tetep, which is a multiple biotic stress tolerant *indica* genotype from Vietnam. In studies conducted at Indian Agricultural Research Institute (IARI), one major ShB QTL *qSBR11-1* from Tetep was functionally characterized and the candidate gene, a novel chitinase gene (LOC\_Os11g47510), for sheath blight tolerance was identified in the QTL region. The QTL *qSBR11-1* was introgressed into the background of 'Improved Pusa Basmati 1' by marker-assisted backcrossing (MAB). In another study, the sheath blight tolerance of the line Pusa 6B, the Basmati quality maintainer line of the popular superfine aromatic rice hybrid Pusa RH10, was enhanced by introgressing three ShB resistance QTLs (*qSBR11-1, qSBR11-2* and *qSBR7-1*) from Tetep by MAB.

The resistance reaction of a genotype may vary depending on the strain of the pathogen used. Screening experiments conducted at the National Rice Research Institute (NRRI) using the local strains of the pathogen has shown that international check genotypes for ShB tolerance like Jasmine 85 and TeQing are susceptible to the



Chr. No.	QTL	Resistant parent	Susceptible parent	Mapping population
5	qShb5.1	RP 2068-18-3-5	TN1	RIL
7	qshb7.3	ARC10531	BPT-5204	BC1F2
9	qshb9.2	ARC10531	BPT-5204	BC1F2
9	qShB9-2	Jasmine 85	Lemont	RIL
9	qSBR-9	Jarjan	Koshihikari	BC2F3 (BIL)
1	<i>qSBR1-1</i>	Tetep	HP2216	RIL
	qSBR1-1	Tetep	HP2216	RIL
7	qSBR7-1	Tetep	HP2216	RIL
	qSBR7-1	Tetep	HP2216	RIL
8	qSBR8-1	Tetep	HP2216	RIL
11	qSBR11-1	Tetep	HP2216	RIL
11	qSBR11-2	Tetep	HP2216	RIL
11	qSBR11-3	Tetep	HP2216	RIL
11	qSB-11LE	Lemont	Yangdao	NIL
1	-	Pecos	Rosemont	F2
9	qShB9-2	Jasmine 85	Lemont	RIL
9	qSB-9Tq	Lemont	Teqing	CSSLs
8	Qsh8a	Teqing	Lemont	RIL
8	Qsh8b	Teqing	Lemont	RIL
9	Rsb-2(t)	A Mutant	Shuhui 881	-
1	<i>qSB-1</i>	Lemont	Teqing	RIL
3	qSB-9	Lemont	Teqing	RIL
5	qSB-3	WSS2	Hinohikari	BC1F1
2	Rsb1	4011	XZX19	F2
11	qSBR-2	Jingxi 17	Zhaiyeqing 8	DH
2	QSbr2a	Lemont	Teqing	NIL
3	QSbr3	Lemont	Teqing	NIL
2	<i>qSB-2</i>	Jasmine 85	Lemont	F2
3	<i>qSB-3</i>	Jasmine 85	Lemont	F2
7	<i>qSB-7</i>	Jasmine 85	Lemont	F2
9	qSB-9-1	Jasmine 85	Lemont	F2
9	qSB-9-2	Jasmine 85	Lemont	F2
11	<i>qSB-11</i>	Jasmine 85	Lemont	F2
1	QRh1	Jasmine 85	Lemont	RIL
9	Qsbr3a	Teqing	Lemont	F4 Bulk
	Qsbr9a	Teqing	Lemont	F4 Bulk

### Table 3. List of reported QTLs for sheath blight tolerance.

Adapted and updated from Srinivasachary et al. (2011).



local strains. Only two genotypes, Tetep and CR 1014, a variety released from ICAR-NRRI, showed consistent moderate resistant phenotype for sheath blight. Conventional breeding has been less effective for the development of ShB tolerant genotypes because of the polygenic nature of the trait. In the segregating generations of the crosses made at ICAR-NRRI, using CR 1014 as the donor for ShB tolerance, selection of superior recombinants has been difficult since ShB tolerance has tight linkage with plant height. A novel ShB QTL on chromosome 1 was identified from an  $F_{2:3}$  population derived from the cross Swarna Sub1 x CR 1014, which need to be fine mapped and its effects in different genetic backgrounds need to be validated.

# 4. BROWN PLANTHOPPER (*NILAPARVATA LUGENS* STÅL) RESISTANCE

Brown planthopper (BPH) (Nilaparvata lugens Stål) is one of the most destructive insect-pests of rice. Besides affecting the rice crop directly, it also serves as a vector that transmits rice grassy stunt virus and ragged stunt virus. The host resistance of rice against BPH was first reported in the variety Mudgo and the first BPH resistance gene (BPH 1) was identified from the same in 1967. After that 31 more genes have been discovered (Table 4) besides several QTLs from the gene pool of cultivated and wild rice (Deen *et al.* 2017). They are mapped to five of the 12 chromosomes (3, 4, 6, 11, and 12) of rice (Cheng et al. 2013). Among those, only 17 genes (BPH1, BPH2, BPH6, BPH9, BPH12, BPH14, BPH15, BPH17, BPH18, BPH19, BPH25, BPH26, BPH27, BPH28, BPH29, BPH30 and BPH32) have been fine-mapped and seven of them (BPH14, BPH17, BPH18, BPH26, BPH29, BPH9 and BPH32) have been cloned and characterized (Jena et al. 2017). Among the cloned genes BPH 9 and BPH 26 turned out to be the same gene (LOC Os12g37280), and the locus IDs for BPH 17 and BPH 18 have not been yet assigned. However, almost all the identified resistance genes are biotype/ population specific and do not provide strong resistance to other BPH biotypes/populations. Hence, search for broad-spectrum resistance should continue besides taking efforts for pyramiding multiple combinations of genes and understanding the detailed molecular mechanisms involved therein.

A series of BPH tolerant varieties (e.g. IR26, IR36, IR50 and IR72) have been developed and released from the IRRI since the 1970s, by transferring BPH resistance genes in the background of elite susceptible cultivars. However, the improved cultivars carrying single resistance gene lose effectiveness due to the evolution of new biotypes and this has become a serious threat to its management in Asia. Pyramiding of BPH resistance genes/QTLs may provide a sustainable means for developing durable resistance against frequently evolving new biotypes. Several studies have been reported for pyramiding of insect resistance genes. The most elaborate work was carried out by Jena et al. (2017) in which the resistance levels of *bph* genes were studied by introgressing them into the genetic background of the variety IR 24. The group has developed 25 NILs with 9 single R genes and 16 multiple R genes combinations. The insect resistance of the NILs, in terms of the level of antibiosis was assessed. It was found that NILs pyramided with multiple *bph* genes were having



S. No.	Resistance gene	Source
1	Bph1	Mudgo, CO22 (IT 000588), TKM6, Milyang30, Milyang34 (IT 006216), Nampungbyeo, Chilseongbyeo, Andabyeo, Kanto PL4 (IT173362), Cheongcheongbyeo, Changsongbyeo, Baekunchalbyeo, IR26 (IT001886), IR28 (IT001892), IR29 (IT001893), IR30 (IT001899), Hangangchalbyeo, Yeongpungbyeo, Namyeongbyeo, Gayabyeo, Samgangbyeo, Namcheonbyeo, MTU15, IR26, IR28, IR29, IR30, IR34, IR44, IR45, IR46, IR64 and MGL2
2	bph2	ASD7, ASD9, IR 1154-243, Norin-PL4, Hwacheongbyeo, PTB18, PTB33, H105, Palasithari 601, H5, IR32, IR36, IR38, IR40, IR42, IR48, IR50, IR52, IR54, IR65
3	Bph3	Rathu Heenati, PTB19, Gangala, Horana Mawee, Muthumanikam, Kuruhondarawala, Mudu, Kiriyal, PTB33, IR56, IR58, IR60, IR62, IR68, IR70, IR72, IR74
4	bph4	Babawee, Gambada Samba, Hotel Samba, Kahata Samba, Thirissa, Sulai, VellaiIIlankali, Heenhoranamawee, KuluKuruwee, Lekam Samba, Senawee and IR66
5	bph5	ARC10550
6	Bph6	Swarnalata, O. officinalis (acc.00896)
7	Bph7	T12
8	bph8	Chin Saba, Col. 5 Thailand and Col. 11 Thailand
9	Bph9	Pokkali, Balamee and Kaharamana
10	Bph10	O. australiensis and IR65482-4-136-2-2
11	bph11	O. officinalis, DV85 and IR 54751-2-44-15-24-3
12	Bph12	O. officinalis, O. latifolia, B14 and IR54751-2-34-10-6-2
13	Bph13	<i>O. eichingeri, O. officinalis</i> (acc.00896), acc105159 and IR54745-2-21-12-17-6
14	Bph14	O. officinalis, RI35 and B5
15	Bph15	O. officinalis and B5
16	Bph17	Rathu Heenati
17	Bph18	O. australiensis and IR65482-7-216-1-2
18	bph19	AS20-1
19	Bph20	O. minuta (acc. 101141), IR71033-121-15 and ADR 52
20	bph21	ADR52, O. minuta (acc. 101141) and IR71033-121-15
21	Bph22	IR 75870-5-8-5-B-2-B and IR 75870-5-8-5-B-1-B
22	Bph23	IR 71033-121-15
23	bph24	IR 73678-6-9-B
24	Bph25(t)	ADR52
25	Bph26(t)	ADR52
26	Bph27	GX2183
27	Bph28(t)	DV85
28	Bph29	RBPH54 (introgression from O rufipogon)
29	Bph31	CR2711-76
30	Bph32	PTB33

Adapted and updated from Ali and Chowdhury (2014).



more level of antibiosis compared to NILs with single *bph* gene. The study throws significant inroads into the concept of R gene deployment in which different *bph* gene/gene combinations can be used in different geographical areas depending on the biotype prevalent in the region.

Deen et al. (2017) reported the occurrence of multiple loci instead of a single recessive gene (reported earlier) conferring resistance to the insect in case of *bph5*. They identified five QTLs *qBphDs6*, *qBphNp1*, *qBphNp12*, *qBphDw3* and *qBphDw8* associated with BPH (biotype 4) resistance in ARC10550. The two major QTLs *qBphDs6* for damage score and *qBphDw8* for days to wilt were important for further investigation and use in the breeding programme. Pyramiding of BPH resistance genes, *Bph1* and *Bph2*, has been successfully achieved by marker-assisted breeding (Sharma et al. 2004).

At ICAR-NRRI, several landraces showing a very high degree of resistance were used for breeding varieties resistant to BPH. The breeding lines CR 3005-77-2 (Samba Mahsuri/Salkathi), CR 3006-8-2 (Pusa 44/Salkathi), CR 3005-230-5 (SambaMahsuri/Salkathi), CR 2711-76 (Tapaswini/Dhobanumberi) were found to be promising in planthopper screening trials of AICRIP, 2011 and 2012. Molecular mapping of resistance genes/QTLs from these two landraces-Salkathi and Dhobanumberi is underway. Two QTLs designated as *qBph4.3* and *qBph4.4* were identified from Salkathi landrace among which *QBph4.3* is novel (Mohanty et al., 2017). Transfer of these two QTLs into two popular susceptible varieties Naveen and Pooja are in progress. Recently, Prahlada et al. (2017) at IRRI identified a single dominant gene, *BPH31* on the long arm of chromosome 3 in CR2711-76.

## 5. YELLOW STEM BORER (SCIRPOPHAGA INCERTULAS) TOLERANCE/RESISTANCE IN RICE

Yellow stem borer is a major threat to rice production in tropical and subtropical rice-growing areas. Lack of availability of an effective source of resistance to this insect in primary gene pool poses a challenge in the study and improvement of this trait. The complex inheritance pattern and screening methodologies for resistance create further complications. In absence of any significant report of studies related to YSB resistance in literature, the works carried out at ICAR-NRRI and other institutes of India are discussed. Unlike the four other biotic stresses mentioned above, comprehensive molecular studies for identification of genes and QTLs conferring resistance to YSB are not available. Most of the studies are confined to classical genetic studies.

Efforts to introgress YSB tolerance in the elite genetic background started immediately after the establishment of the institute. Screening studies conducted during 1950's at ICAR-NRRI resulted in the identification of YSB tolerant genotypes viz., TKM6, Slo-12, CB-1, MTU 15, Tepa-1, ADT-14 and JBS 1638. Among these,



TKM6 was extensively used in the resistance breeding programme at the institute. Three YSB tolerant varieties were released from ICAR-NRRI using TKM6 as the donor. The varieties are, Ratna (TKM6 x IR 8) which is highly tolerant to YSB especially at the vegetative stage, Saket 4 (sister selection of Ratna) and CR138-928 (Jaya x TKM6). Other popular YSB tolerant varieties released from ICAR-NRRI include Vijaya (T90 x IR8), Supriya (IR8//GEB24/T(N)1), Dharitri (Pankaj x Jagannath) and Panidhan (CR151-79 x CR1014). Mutation breeding was also attempted to develop YSB tolerant lines; a mutant line of Tainan3 was released in 1980 as the variety Indira (CR MUT 587-4) which possess a fair degree of YSB tolerance in addition to tolerance to blast and BB. Besides NRRI, two more varieties, Sasyasree and Vikas with a moderate level of resistance to YSB were released in India using TKM6 as the donor source. YSB resistance was mapped by RAPD markers from a cross of Co43 x W1263. Though the high yielding rice varieties enlisted above are moderately resistant to YSB, no rice variety truly resistant to YSB has yet been developed.

Since gene(s) for resistance to YSB has not been found in the primary gene pool of rice efforts were made to incorporate alien genes from wild species belonging to the secondary gene pool, which are reservoirs of such traits. Wild rice germplasm has been screened against YSB. *O. brachyantha, O. officinalis, O. ridleyi* and *O. coarctata* were found to be resistant/tolerant against the pest. Subsequently, backcross population of *O. sativa* cv. Savitri/*O. brachyantha* was developed to transfer YSB resistance to the cultivated rice (Behura et al. 2011). The cytogenetic analysis of the chromosomal variants lead to the development of monosomic alien addition lines (MAALs). Of the 8 MAALs screened, MAAL 11 was found to be moderately resistant to YSB.

## 6. STATUS OF UTILIZATION OF WILD GENE POOL FOR BIOTIC STRESS TOLERANCE

The genus *Oryza* comprises of several wild species besides the two cultivated species *Oryza sativa* (Asian rice) and *Oryza glaberrima* (African rice) (Table 5). These wild relatives of cultivated rice are found to be grown naturally in different ecologies around the world. The term species complex is used "for a group of species where distinct taxonomic keys are lacking and the categorization to species or subspecies level is rather arbitrary" (Vaughan 2005). Four major species complexes of *Oryza* were identified which were designated as *O. sativa* complex (contains AA genome), *O. officinalis* complex (comprises diploid and allotetraploid species of BB, CC, DD or EE genomes), *O. granulata* complex (GG genome) and *O. ridleyi* complex (allotetraploids of HH and JJ or KK genome). There is also a prominent outgroup consisting of a lone species *O. brachyantha* (FF genome). These wild relatives are considered as virtually untapped reservoir of agronomically important genes especially for genes conferring resistance to biotic and abiotic stresses.



## Table 5. Different species of genus *Oryza* and their useful traits for biotic stress tolerance.

Oryza species	Chr. No.	Genome	Origin	Useful traits
O. sativa complex				
O. rufipogon	24	AA	Tropical Asia	Rresistance to BB and tolerance to tungro
O. nivara	24	AA	Tropical Asia	Resistance to grassy stunt virus and BB
O. longistaminata	24	AA	Africa	Resistance to BB
O. barthii	24	AA	Africa	-
O. meridionalis	24	AA	Tropical Australia	-
O. glumaepatula	24	AA	South and Central America	-
O. officinalis complex				
O. punctata	24, 48	BB, BBCC	Africa	Resistance to BPH
O. minuta	48	BBCC	Philippines and Papua New Guinea	Resistance to sheath blight, blast, BB, BPH
O. malampuzhaensis	48	BBCC	Southern India	Resistance to BB
O. officinalis	24	CC	Tropical Asia	Resistance to BPH, WBPH and GLH
O. rhizomatis	24	CC	Sri Lanka	-
O. eichingeri	24	CC	South Asia and East Africa	Resistance to BPH, WBPH and GLH
O. latifolia	48	CCDD	South America	Resistance to BPH
O. alta	48	CCDD	South America	Resistance to stem borer
O. grandiglumis	48	CCDD	South America	-
O. australiensis	24	EE	Tropical Australia	Resistance to BPH and blast
O. granulata complex				
0. granulata	24	GG	Southeast Asia	-
O. meyeriana	24	GG	Southeast Asia	-
O. ridleyi complex				
O. longiglumis	48	ННЈЈ	Indonesia	Resistance to blast and BB
O. ridleyi	48	HHJJ	South Asia	Resistance to blast, BB and stemborer
O. schlechteri	24	HHKK	Papua New Guinea	-
O. coarctata	48	HHKK	India	-
Outgroup				
O. brachyantha	24	FF	Africa	Resistance to yellow stem borer



# 7. MAPPING OF GENES/ QTLS FROM WILD RICE AND THEIR UTILIZATION

The rice breeders have mostly preferred hybridization among the members of cultivated gene pool like *indica-indica, japonica-japonica, indica-japonica, indica-t*ropical *japonica* in their regular breeding programmes. Utilization of wild species remained limited although in several cases, genetic variability for target agronomic traits were lacking in the primary gene pool. The wild species of rice have been utilized as a valuable source of genes for tolerance to various biotic (Table 6) and abiotic stresses. Several major genes for resistance to brown planthopper (BPH), white backed plant hopper (WBPH), gall midge, bacterial blight (BB), sheath rot and leaf/neck blast have been identified from them. Several alien introgressed lines developed using wild *Oryza* as the donor has been released in different countries (Brar and Singh 2011).

The transfer of wild genes in cultivated rice depends on multiple factors like the inheritance pattern of the trait (quantitative/qualitative or monogenic/oligogenic/polygenic), phylogenetic relationship of cultivated and wild species and the presence of reproductive barriers. Several pre- and post-fertilization barriers create difficulty in hybridization of wild and cultivated rice. The transfer of desired genes or QTLs from wild rice is difficult as the wild species are associated with several weedy traits like grain shattering, low grain yield/quality and unwanted plant types. Along with advancements in plant tissue culture techniques especially embryo rescue and protoplast fusion, wild species are increasingly being used in gene transfer. Cytogenetic techniques along with the availability of cross-transferrable markers derived from genome sequencing projects have created further opportunities for precise transfer of genomic regions from wild species.

Among several species of *O. sativa* complex, wild introgression lines for biotic stress tolerance have been developed mostly for resistance to bacterial blight. Three important genes for BB resistance have been mapped from the members of this species complex namely *Xa30* (*t*) from *O. nivara*, *Xa23* from *O. rufipogon* and *Xa21* from *O. longistaminata*. These genes have further been utilized worldwide for rice breeding.

Ten distinct species are found in *O. officinalis* complex which are either diploid or allotetraploid. The basic genomic groups are BB, CC, DD or EE. Two C- genome species have mostly been used, namely *O. officinalis* and *O. eichingeri*. Many of the introgression lines derived from *O. officinalis* complex confers resistance to BPH besides genes for resistance to WBPH, BLB and sheath rot. In Vietnam, four *O. officinalis* derived BPH resistance lines have been released as varieties (Brar and Singh, 2011). *O. eichingeri* have also been used for transfer of BPH resistance genes to cultivated rice. Although interspecific hybrids were derived between *O. sativa* and tetraploid wild species *O. minuta*, *O. punctata* and *O. malampuzhaensis;* development of advanced introgression lines was only possible with *O. minuta* for transferring resistance against BPH, BLB and blast. Among the three species with CCDD genome *O. latifolia*, *O. grandiglumis* and *O. alta*, the third one is yet to be utilized in rice breeding. However, introgression lines were derived from the rest two species. BPH,



Wild species	Trait	Genes/QTL
O. rufipogon	BB	Xa23
O. nivara	BB	Xa30(t)
O. longistaminata	BB	Xa21
O. officinalis	BPH	Bph6, Bph11, Bph13(t), Bph15
	BB	Xa29
O. eichingeri	BPH	Bph13
O. minuta	BPH	Bph20(t) and $Bph21(t)$
	BB	Xa29
	Blast	Pi9(t).
O. latifolia	BPH	Bph12
O. australiensis	BPH	Bph10, Bph18
	Leaf and neck blast	Pi40(t)

Table 6. List of genes/ (	TLs identified from wild rice for biotic stress resistance.
Table 0. List of genes/	2 1 Lis facilitation from what fice for brothe stress resistance.

WBPH and BLB resistant lines have been developed by transfer of genes from *O. latifolia*. From backcross progeny lines of *O. sativa*  $\times$  *O. grandiglumis*, although no genes for stress tolerance were transferred, QTLs for yield contributing traits have been mapped successfully. *O. australiensis* (EE) derived introgression with resistance to BPH and leaf blast have been developed. Several important genes like *Bph10*, *Bph18* and *Pi40* (*t*) have been tagged from these lines.

Introgression line development from *O. ridleyi* and *O. granulata* complex, as well as *O. brachyantha* for biotic stress tolerance especially for the stresses considered in this book chapter, is still lacking. However, MAAL lines with tolerance to many of these stresses have been successfully developed by several researchers.

#### 8. KNOWLEDGE GAPS AND RESEARCH NEEDS

Except for sheath blight and YSB, for all the pathogens and insects discussed here, several major genes conferring resistance have been identified, fine mapped and few of them have been cloned (Fig. 1). Many of them are also in use by the breeders for developing disease resistant cultivars. Despite the reasonably good amount of knowledge generated and genomic resources developed, breeders still find difficulty in their judicious utilization in marker-assisted selection. Out of so many genes known for disease resistance, lack of highly reproducible functional markers for most of them creates troubles in their appropriate utilization. There is a need for mega-scale allele mining among the large pool of susceptible and resistant cultivars. Such a search should go beyond the cultivated species and must include multiple accessions of wild species. Rather than targeting only one SNP, most appropriate haplotypes must be identified after precise phenotyping.

Despite being the storehouse for genes of resistance to various biotic stresses, utilization of genes and alleles from wild species is still very limited. Precise transfer of genes from wild species avoiding linkage drag is quite difficult till now for most of the



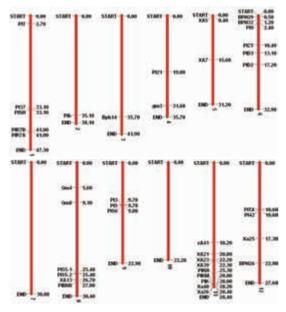


Fig. 1. Chromosomal location of cloned biotic stress resistance genes in rice

breeders. Lack of availability of genomic resources especially genome-wide markers for wild species creates a major bottleneck for this. However with the availability of genome sequences for more number of wild species (genome sequence is now available for eight wild and two cultivated species of *Oryza*) such bottlenecks are expected to be removed very soon.

For many biotic stresses, despite sincere efforts, it has not become possible till date to assign resistance function to a single gene. However, QTLs with various level of tolerance or resistance have been mapped. Although many

of these QTLs are genotype specific, some major QTLs were found to work across populations. Precise mapping of those QTLs and their subsequent utilization in large scale is expected in near future.

With large numbers of genes or QTLs being mapped, the question arises about identifying the appropriate combinations of genes or QTLs for pyramiding in a single background. Different genes or QTLs conferring resistance to same stress have different mechanisms of actions. Identifying their appropriate combinations which will confer maximum and durable resistance without any adverse effect on plant growth and development is need of the hour. All the discovered genes or QTLs may be pyramided in various combinations and tested across different growing environments. Some efforts in this direction have already been initiated (Jena et al. 2017) which needs to be strengthened further.

All the research on resistance to biotic stresses will fail if there is any gap in phenotyping methods. With increasing needs for mega-scale phenotyping for biotic stress resistance, development of an easy yet effective protocol to clearly distinguish the escapes from true resistance is the need of the time.

### 9. WAY FORWARD

The primary requirement for breeding tolerance to biotic stresses is availability of precise phenotyping standards which will work across locations and can clearly distinguish resistance from escapes. Whenever such phenotyping standards are



available, the phenotyping for those biotic stresses should be carried out in large scale utilizing the network mode of AICRP or international trials of IRRI. This will help in keeping track of evolution of new pathogens or insect biotypes and search for their corresponding resistance source. The effective resistant QTLs or genes identified though biparental mapping approaches should be supplemented with genome wide association mapping to identify genes/QTLs which will work across populations. After discovery of any gene or QTL, its optimum pyramiding combinations should be worked out with reported genes or QTLs. Till date the major target of scientists working on host plant resistance remains limited to search for R-genes in host genomes. With advancements in genome sequencing, the scope for utilization of genome sequences of both pest and host for understanding mechanism of resistance as well as breakdown of resistance have increased. For identification of functional markers, identification of superior functional haplotypes of resistance genes from both wild and cultivated species is highly required. Prediction of R-genes from genomes of wild species through bioinformatics approaches and their validation will also be useful. It is important to note that stable and durable resistance genes present in wild rice are yet to be exploited in large scale. There is urgent need for inclusion of more numbers of wild species in breeding programmes of rice through pre-breeding and marker assisted selection for their judicious utilization in resistance breeding of rice.

#### References

- Ali MP, Chowdhury TR (2014) Tagging and mapping of genes and QTLs of *Nilaparvata lugens* resistance in rice. Euphytica 195(1):1-30.
- Behura N, Sen P and Kar MK (2011) Introgression of yellow stem borer (*Scirpophaga incertulus*) resistance genes into cultivated rice (*Oryza* sp.) from wild species. The Indian Journal of Agricultural Sciences 81(4):359-362.
- Brar DS and Singh K (2011) Oryza. In: Kole C (ed) Wild crop relatives: genomic and breeding resources, cereals. Springer, Berlin pp. 321-365.
- Chen ZX, Zhang YF, Feng F, Feng MH, Jiang W, Ma YY, Pan CH, Hua HL, Li GS, Pan XB and Zuo SM (2014) Improvement of japonica rice resistance to sheath blight by pyramiding qSB-9TQ and qSB-7TQ. Field Crops Research 161:118-127.
- Cheng X, Zhu L and He G (2013) Towards understanding of molecular interactions between rice and the brown planthopper. Molecular Plant 6(3):621-634.
- Dash AK, Rao RN, Rao GJ, Verma RL, Katara JL, Mukherjee AK, Singh ON and Bagchi TB (2016) Phenotypic and Marker-Assisted Genetic Enhancement of Parental Lines of Rajalaxmi, an Elite Rice Hybrid. Frontiers in Plant Science 7(2016):1005.
- Deen R, Ramesh K, Padmavathi G, Viraktamath BC and Ram T (2017) Mapping of brown planthopper [*Nilaparvata lugens* (Stål)] resistance gene (bph5) in rice (*Oryza sativa* L.). Euphytica 213(2):35.
- Ellur RK, Khanna A, Bhowmick PK, Vinod KK, Nagarajan M, Mondal KK, Singh NK, Singh K, Prabhu KV and Singh AK (2016) Marker-aided Incorporation of Xa38, a Novel Bacterial Blight Resistance Gene, in PB1121 and Comparison of its Resistance Spectrum with xa13+ Xa21. Scientific Reports 6:29188.



- Hittalmani S, Parco A, Mew TV, Zeigler RS and Huang N (2000) Fine mapping and DNA markerassisted pyramiding of the three major genes for blast resistance in rice. Theoretical and Applied Genetics 100(7):1121-1128.
- Jena KK, Hechanova SL, Verdeprado H, Prahalada GD and Kim SR (2017) Development of 25 near-isogenic lines (NILs) with ten BPH resistance genes in rice (*Oryza sativa L.*): Production, resistance spectrum, and molecular analysis. Theoretical and Applied Genetics 130(11):2345-2360.
- Li W, Zhu Z, Chern M, Yin J, Yang C, Ran L, Cheng M, He M, Wang K, Wang J and Zhou X (2017) A natural allele of a transcription factor in rice confers broad-spectrum blast resistance. Cell 170(1):114-126.
- Liang Z, Wang L and Pan Q (2016) A new recessive gene conferring resistance against rice blast. Rice 9(1):47.
- Mohanty SK, Panda RS, Mohapatra SL, Nanda A, Behera L, Jena M, Sahu RK, Sahu SC and Mohapatra T (2017). Identification of novel quantitative trait loci associated with brown planthopper resistance in the rice landrace Salkathi. Euphytica 213(2):38.
- Pinson SR, Oard JH, Groth D, Miller R, Marchetti MA, Shank AR, Jia MH, Jia Y, Fjellstrom RG and Li Z (2008) Registration of TIL: 455, TIL: 514, and TIL: 642, three rice germplasm lines containing introgressed sheath blight resistance alleles. Journal of Plant Registrations 2(3):251-254.
- Pradhan SK, Nayak DK, Mohanty S, Behera L, Barik SR, Pandit E, Lenka S and Anandan A (2015) Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna. Rice 8(1):19.
- Pradhan SK, Nayak DK, Pandit E, Behera L, Anandan A, Mukherjee AK, Lenka S and Barik DP (2016) Incorporation of bacterial blight resistance genes into lowland rice cultivar through marker-assisted backcross breeding. Phytopathology 106(7):710-718.
- Prahalada GD, Shivakumar N, Lohithaswa HC, Sidde Gowda DK, Ramkumar G, Kim S R, Ramachandra C, Hittalmani S, Mohapatra T and Jena KK (2017) Identification and fine mapping of a new gene, BPH31 conferring resistance to brown planthopper biotype 4 of India to improve rice, *Oryza sativa* L. Rice 10:41.
- Ray S, Singh PK, Gupta DK, Mahato AK, Sarkar C, Rathour R, Singh NK and Sharma TR (2016) Analysis of *Magnaporthe oryzae* genome reveals a fungal effector, which is able to induce resistance response in transgenic rice line containing resistance gene, Pi54. Frontiers in Plant Science 7:1140.
- Reddy JN, Baraoidan MR, Bernardo MA, George ML and Sridhar R (1997) Application of markerassisted selection in rice for bacterial blight resistance gene, Xa21. Current Science 73 (10): 873-875.
- Sharma PN, Torii A, Takumi S, Mori N and Nakamura C (2004) Marker-assisted pyramiding of brown planthopper (*Nilaparvata lugens* Stål) resistance genes Bph1 and Bph2 on rice chromosome 12. Hereditas 140(1):61-69.
- Sharma TR, Rai AK, Gupta SK, Vijayan J, Devanna BN and Ray S (2012) Rice blast management through host plant resistance: Retrospect and prospects. Agricultural Research 1(1):37-52.
- Singh AK, Gopalakrishnan S, Singh VP, Prabhu KV, Mohapatra T, Singh NK, Sharma TR, Nagarajan M, Vinod KK, Singh D and Singh UD (2011). Marker assisted selection: a paradigm shift in Basmati breeding. Indian Journal of Genetics and Plant Breeding 71(2):120-128.



- Srinivasachary, Willocquet L and Savary S (2011) Resistance to rice sheath blight (*Rhizoctonia solani* Kuhn)[teleomorph: *Thanatephorus cucumeris* (AB Frank) Donk.] disease: current status and perspectives. Euphytica 178:1-22.
- Vaughan DA, Kadowaki KI, Kaga A and Tomooka N (2005) On the phylogeny and biogeography of the genus Oryza. Breeding Science 55(2):113-122.
- Wang Y, Pinson SRM, Fjellstrom RG and Tabien RE (2012) Phenotypic gain from introgression of two QTL, qSB9-2 and qSB12-1, for rice sheath blight resistance. Molecular Breeding 30(1):293-303.
- Zuo SM, Zhang YF, Chen ZX, Jiang W, Feng MH and Pan XB (2014) Improvement of rice resistance to sheath blight by pyramiding QTLs conditioning disease resistance and tiller angle. Rice Science 21(6):318-326.\*



## Enhancing Input Use Efficiency in Directseeded Rice with Classical and Molecular Breeding

### A Anandan, J Meher, RP Sah, S Samantaray, C Parameswaran, P Panneerselvam, SK Dash, P Swain, Prabhukarthikeyan SR, M Annamalai and G Kumar

## SUMMARY

The dwindling freshwater resource and rising cost of rice cultivation have lead to the debate of ways to enhance net return from rice cultivation for ever increasing food demand for growing population. A resource use efficient system of rice cultivation with water use efficient varieties is very much needed to improve the water productivity in agriculture. Dry direct-seeded/aerobic rice system is a strategy that ensures water use efficiency in rice crop to cope with the looming problem of water scarcity. To achieve high yield, a suitable variety for this condition needs to have tolerance for moisture stress, input use efficiency, weed competitiveness, and location-specific pest and disease resistance. Recent developments in the field of dry direct seeded/ aerobic rice, yield component and nutrient use efficient QTLs and breeding strategies are discussed in this chapter to improve grain yield.

## **1. INTRODUCTION**

Puddled transplanted rice (TPR) system with stagnant water irrigation system is reported to increase the productivity more than other systems of cultivation, wherein restrained weed growth, trouble-free seedling establishment and increased nutrient availability (e.g. iron, zinc, and phosphorus) are advantages of the TPR. However, the hefty sum of water, more input, energy and time are required for TPR and these factors make rice cultivation more expensive and less profitable. Thus, improving the crop productivity and economic security to farmers with an alternate system of rice production is very much necessary. Therefore, shifting of rice cultivation from TPR to dry direct-seeded rice (DSR) is necessary to make income from rice cultivation sustainable. Additionally, water has become a precious commodity in the era of modern agriculture, but groundwater tables are started falling, and wells are going dry. Even though, the green revolution met the food demand for our country, there is a demand to extract water for irrigation has also started simultaneously. Further, millions of farmers started to drill deeper irrigation bore wells to expand their harvests and resulting in running down of groundwater table and wells in 20 countries, including three countries that together produce half the world's grain viz., India, China, and the United States (Somanathan 2010). Consequently, the increase in production of cereal grain came from irrigated land that has 40 percent share. But this inflated production from irrigated land will burst when the aquifer gets depleted. Therefore, the decline in



the supply of irrigation water is of great concern. In India, three fifths of grain harvest come from irrigated land (Dick and Rosegrant 2009), where over-pumping of water for irrigation is predominant. The states such as Punjab and Haryana witnessed falling water table and green revolution achieved in India was based on water mining. In Punjab, the number of tube wells has been increased from 1.9 lakhs in 1970 to 14 lakhs in 2010, indicates that India's food production is water based and may burst (Garg and Hassan 2007) any time.

The rural-urban competition for water resource is gradually intensifying in India and was evident that water needs of Chennai depend on popular tank-truck industry. This stiff competition for water in urban areas of India does not favours the farmers, as producing 1 kg of rice requires ~3,500 L of water. The greatest impact on water consumption for urban needs is likely to continue well into the middle of the twentyfirst century (FAO 2006). Consequently, in a point, water scarcity would translate into food scarcity. Thus, to reduce the level of water dependency and to improve the water productivity in rice cultivation, water use efficient rice genotypes are one of the viable options. Aerobic rice is one such extensive water-saving technology for rice cultivation/production compared to other production methods. Aerobic rice systems use less water than conventional flooded rice, by using suitable rice varieties capable of responding well to reduced water inputs in non-puddled and non-saturated soils (Atlin et al. 2006). Additionally, continuous standing water in paddy field favours more nitrogen leaching loss than either in field capacity (aerobic) or alternate wetting and drying. Moreover, application of recommended and/or excessive fertilizer is not fully utilized by the plant. The unutilized portion escapes into environment through runoff, leaching, ammonia volatilization, and N<sub>2</sub>O into atmosphere and water systems (Zhu and Chen 2002). On the other hand, around 80–90% of applied P is also not easily available to plant and transforms into other forms and eventually lost into the water body causing eutrophication (Schindler et al. 2016). This detrimental impact needs to be addressed by increasing the Nutrient Use Efficiency (NUE) of rice in concert with other management practices. It is therefore critical that strategies for genetic improvement of NUE needs to be undertaken for cultivating rice with less water and higher NUE to maximize biomass and yield. Reducing the cost of inputs would be one of the prime solutions to increase farm income in rice cultivation. Additionally, the genetic potential of yield could be achieved through proper nutrient and weed management. This chapter addresses the major avenues for increasing the productivity and profitability of farmers by increasing the NUE under DSR, where labor workforce shortage is highly prevalent in eastern India.

## 2. PROBLEMS OF CONVENTIONAL PUDDLED TRANSPLANTED RICE

The anticipated climate change, shortage of water and natural resources and shortage of man power are likely to be major researchable issues in future. Under such circumstances, direct-seeded rice system can mitigate the adverse situation for rice cultivation and minimizes the negative impacts of TPR by reducing the labor



requirement and, increasing water and NUE. In Asia, the practice of dry seeding is popular and extensively adopted in rainfed lowlands, uplands, and flood-prone areas with an area of 26% in south Asia and 28% in India. Globally, 23% of rice is direct seeded, as wet seeding is being the common practice and predominantly practiced in irrigated areas of Australia, Brazil, Chile, Cuba, France, Italy, Japan, Korea, Malaysia, the Philippines, Thailand, Russia, Sri Lanka, Vietnam, and in some parts of Iran, due to shortage of skilled labour, high labour cost and availability of mechanised system (Mahender et al. 2015). Thus, DSR system of cultivation should be encouraged, as agricultural labor workforce is reducing relative to the total workforce in India. It is reported that ~30 million agricultural labor workforces in India was reduced as compared to 2004-05 (FICCI report). The states of Uttar Pradesh (28%), Odisha (14%), Bihar (12%) and West Bengal (12%) contributed on an average of 16.5% reduction in agricultural workforce in India.

DSR system showed substantial water saving. Trials conducted in Haryana by adopting zero or reduced till system resulted in good grain vield comparable with TPR under less water with more water productivity and greater net profit. Moreover, it increases net return, efficiency in water and fertilizer use (Anandan et al. 2015). The varieties released during and after green revolution has ability to utilize less than 50% of applied fertilizer. These varieties combined with intensive agricultural practices, effects environment through methane emission and eutrophication of water bodies. Therefore, DSR, helps farmers to earn more carbon credits than TPR by mitigating methane emission and provides higher economic returns, saves water and reduces labour requirement. However, no specific varieties possessing all suitable traits of DSR have been developed with nutrient and water use efficiency. Varieties that are necessary for DSR should possess good mechanical strength in the coleoptiles to make easy emergence of the seedlings under crust conditions, weed competitiveness with early seedling vigour, efficient root system to tap soil moisture and nutrients (Fig. 1 and 2), early maturing, photoperiod-insensitive with better drought tolerance and yield stability. Specifically, improving NUE is very much needed in our rice-wheat cropping system in India. Moreover, the consumption of N fertilizer has been increased from 0.06 Mt in 1950-1951 to 10.8 Mt in 2000-01 and P fertilizer has steeply increased from 0.01 Mt to 1.8 Mt in the last 50 years (FAI 2000-2007). Correspondingly, N and P fertilizer contributed around 64% and 78%, respectively in Indian agriculture (Pathak et al. 2010). Further, Pathak et al. (2010) highlighted that states like Tamil Nadu, Gujarat, Haryana, Punjab and West Bengal showed 50% N use efficiency and less than 50% N use efficiency was observed in the states like Bihar, Odisha and Uttar Pradesh. Also, Odisha and Bihar showed minimum P balance (Pathak et al. 2010). Thus, there is a need to increase the N use efficiency and PUE of rice in eastern India to reduce the cost of cultivation in sustainable way and increase the per capita food availability. Developing rice cultivars with improved NUE becomes a prerequisite in protecting the environment by reducing the rate of nutrient loss into the ecosystems. It also reduces input cost and improves rice yield in a sustainable manner, while maintaining soil and groundwater quality. Nutrient use efficient varieties can also be raised in marginal lands where nutrient availability is limited. Therefore, the present breeding program should be prioritized to develop rice varieties with high grain yield under





Fig.1. Variability in rice root system.



Fig. 2. Direct seeded rice genotypes should possess good numbers of lateral and deep roots for absorbing nutrient and water in symbiotic association with AMF and bacteria efficiently from soil.

low-nutrient conditions (Vinod and Heuer 2012). Significant genotypic differences in nitrogen (Singh et al. 1998) and phosphorus (Wissuwa and Ae 2001) use efficiency exist in rice with several mechanisms and morphophysiological traits to sustain their growth. Among the several rice accessions, it is observed that landraces are being superior in nutrient uptake. It is reported that P concentration varied from 0.6 to 12.9 mg P plant" (Wissuwa and Ae 2001). Therefore, the variability exists in the form of morpho-physiological traits related tolerance to P deficiency could effectively be exploited through systematic breeding program to develop cultivars with high NUE and water use efficiency (Ali et al. 2012).

In order to increase the NUE, traits involved in nutrient absorption, transport, utilization and mobilization should be identified and in combination with best management practices it could provide sustainable rice cultivation. On the other hand, role of microorganisms in enhancing the availability of N and P needs to be recognized. Several reports have proved that inoculations of the microbial consortium have been shown

to enhance NUE particularly phosphorus, nitrogen, and carbon in many crop plants. Rice differs from most of the crops, since it is typically cultivated in flooded soil, resulting in aerobic and anaerobic zones within the rice rhizosphere, and preferred by specific physiological groups of microorganisms i.e. aerobic, anaerobic, or facultative (Brune et al. 2000). Hence, it is essential to understand the soil microbes and plant interaction for better input resource management for sustainable rice cultivation. Also, presence of variability in microbial association with plants (Hardoim et al. 2011) provides the possibility of breeding cultivars for specific microbial community (Mahender et al. 2017) to gain NUE. The objectives of this chapter are i) to review the water and nutrient (N and P) use efficiency scenario in rice cultivation of India, and ii) to understand how to improve water and NUE in rice through classical and molecular breeding approaches.



# 3. DRY DIRECT SEEDED/AEROBIC RICE ON WATER USE EFFICIENCY

Dry direct seeded rice refers to a cultivation system in which rice is dry direct seeded in well-tilled levelled fields with the uniform slope under unpuddled conditions. When the crop is cultivated with no standing water throughout the season under a well-aerated condition at field capacity is termed as aerobic rice, occasional water stagnation may occur under rainfed low land condition. Thus, the high proportion of water savings associated with this method compared with conventional rice growing practices has made this method increasingly popular in irrigated areas, where the problem of water shortage occurs (Kumar and Ladha 2011). The affinity of the rice crop with water is universally known. Rice cultivation in puddled fields is well known, technologies such as dry/aerobic and wet direct seeding and alternate wetting and drying (AWD) could be aviable option to produce rice in both irrigated and rainfed rice ecosystems. Aerobic rice is one such extensive water-saving technology for rice, reducing labor requirements, mitigating greenhouse gas emissions, and adapting to climatic risks; and the yield can be compared with that of transplanted rice if the crop is properly managed (Kumar and Ladha 2011).

In Brazil and northern China, aerobic rice is grown commercially in 140,000 ha. In China, temperate aerobic rice cultivars under supplementary irrigation exhibited grain yield of 6 t/ha (Bouman et al. 2005). These varieties need 60% less water than that required for lowland rice and their total water productivity was 1.6-1.9 times higher (Guang-hui et al. 2008). In temperate zone country like the United States, lowland rice varieties were tested under aerobic condition and observed 20-30% yield reduction in high yielding cultivars (7-8 t/ha). The decline in yield under aerobic was due to the reduction in panicles per meter square, spikelets per meter square, poor grain filling, and harvest index. Comparatively, flooded rice had 20% more panicles per meter square, 15% more spikelets per meter square and 13% higher grain filling than aerobic rice (Visperas et al. 2002). Under scanty water supply of 450-650 mm, 4-6 t/ha of grain yield was observed in much drier soil condition against 1300-1500 mm of water used in lowland situation. Other than the merit of efficient use of water, aerobic rice demonstrated better nitrogen use efficiency (George et al. 2001).

The rice that is being grown extensively in the upland ecosystem (direct seeding), showed wide genetic variation for aerobic adaptation in rice germplasms. Several quantitative trait loci (QTL) for grain yield have been reported for both favorable irrigated and unfavorable upland ecology (Venuprasad et al. 2009). However, very few reports have reported the genomic regions responsible for increased aerobic adaptation of rice. Few encouraging reports are available regarding grain yield QTLs found under aerobic condition from International Rice Research Institute (IRRI), Philippines. Venuprasad et al. (2012) reported two closely linked rice microsatellite (RM) markers RM510 and RM19367 located on chromosome 6 were found to be associated with grain yield under aerobic soil conditions, consistently in three genetic backgrounds. The QTL linked to this marker, *qDTY6.1*, was mapped to a 2.2 cM region between RM19367 and RM3805 at a peak LOD score of 32 in the Apo/2\*Swarna



population. The effect of qDTY6.1 was tested in a total of 20 hydrological environments over a period of five seasons and in five populations in the three genetic backgrounds (Apo/2\*Swarna, Apo/IR72, and Vandana/IR72). In the Apo/2\*Swarna population, qDTY6.1 had a large effect on grain yield under favorable aerobic (R<sup>2</sup> d" 66%) and irrigated lowland (R<sup>2</sup> d" 39%) conditions but not under drought stress. Further, they conclude that qDTY6.1 is a large-effect QTL for rice grain yield under aerobic environment and could potentially be used in the molecular breeding of rice for the aerobic environment. So far, no variety has been developed that possesses traits specifically needed to produce high yield under aerobic conditions, particularly for rainfed systems that may be prone to low fertility (Sandhu et al. 2015). Kato et al. (2009) suggested that aerobic rice varieties should possess large numbers of spikelets and sufficient adaptation to aerobic conditions will consistently achieve yields comparable to the potential yield of flooded rice.

Sandhu et al. (2015) identified several promising QTLs that showed large and consistent effects from two mapping populations derived from crosses of Aus276, a drought tolerant *aus* variety, with MTU1010 and IR64, high-yielding *indica* mega-varieties. They have reported that QTLs qGY1.1, qGY6.1, and qGY10.1 were found to be effective in both populations under multiple conditions. On the other hand, several of the QTLs identified for grain yield in their study (qGY1.1, qGY6.1, qGY8.1, qGY9.1, and qGY10.1) were found to be previously reported and consistent across different mapping populations, under different drought severities.

In 2012, Ye et al. (2012) have identified two major QTLs *qHTSF1.1* ( $R^2 = 12.6\%$ ) and *qHTSF4.1* ( $R^2 = 17.6\%$ ), were detected on chromosome 1 and 4, respectively, in BC<sub>1</sub>F<sub>1</sub> and F<sub>2</sub> progeny generated from the cross IR64 x N22. Later in 2015, Ye et al. through fine-mapping validated the effect of *qHTSF4.1* with PCR-based SNP markers. They found that the sequence in the QTL region is highly conserved and large numbers of genes in the same gene family were observed to be clustered in the region. The QTL *qHTSF4.1* consistently increased spikelet fertility in all of the backcross populations (BC<sub>2</sub>F<sub>2</sub>, BC<sub>3</sub>F<sub>2</sub>, BC<sub>3</sub>F<sub>3</sub>, and BC<sub>5</sub>F<sub>2</sub>) and this was confirmed again in 24 rice varieties. Most of the rice varieties with this QTL showed a certain degree of increase in spikelet fertility. In a BC<sub>5</sub>F<sub>2</sub> population with the clean background of IR64, QTL *qHTSF4.1* increased spikelet fertility in rice at the flowering stage. PCR-based SNP markers developed from their study would be useful for QTL introgression and for pyramiding with other agronomically important QTLs/genes through marker-assisted selection.

Several research institutes/universities across India started developing rice varieties that consume less water for rice cultivation with more water use efficiency. ICAR-NRRI, too involved in aerobic rice research and developed 9 aerobic (water use efficient) rice varieties and released through Central Sub-Committee on Seed Standards, Notification and Release (CVRC) and State Variety Release Committee (SVRC). Three aerobic rice varieties Anagha (ARB 6), MAS 26 and MAS 946-1 were released from

the University of Agricultural Sciences (UAS), GKVK, Bangalore for the state of Karnataka. Performance of these varieties was found to be well under aerobic with fair degree of drought tolerance. These genotypes need to be irrigated at the intervals of 5 to 7 days and irrigation can be skipped in the event of rainfall. The grain yield potential of these lines was 7.0 t/ha in station trials at UAS, Bangalore, while in farmers' field it recorded with an average yield from 3.0 to 5.0 t/ha (Shashidhar 2012).

Efforts have been taken to identify QTLs responsible for grain yield under aerobic/ dry direct seeded rice in India. However, limited reports are available in relation to QTLs. Recently, Sandhu et al. (2013) have mapped 35 QTLs associated with 14 traits on chromosomes 1, 2, 5, 6, 8, 9, and 11 in MAS ARB25 x Pusa Basmati 1460 and 14 QTLs associated with 9 traits were mapped on chromosomes 1, 2, 8, 9, 10, 11, and 12 in HKR47 × MAS26 from CCS Haryana Agricultural University, Hisar. The QTLs, qGY8.1 (R<sup>2</sup> value of 34.0%) and qGY2.1 (R<sup>2</sup> value of 22.8%) of MAS ARB25 × Pusa Basmati 1460 population and QTL qGY2.2 (R<sup>2</sup> value of 43.2%) of HKR47 × MAS26 population were found promising for grain yield under aerobic condition. Among the three yield QTLs, qGY8.1 showed an increased stable effect over two different years and combined over two years with 26.6% yield improvement. Further, the authors highlighted the QTL hotspot region at 25.1cM segment between RM589 and RM314 on chromosome 6 affects different root (RV, RT, and FRW) and shoot (FSW and DSW) traits under aerobic conditions of two mapping populations (MAS ARB25 x Pusa Basmati 1460 and HKR47 × MAS26). This region is found to be co-localized with *qDTY6.1* region reported by Venuprasad et al. (2011). It was found to be associated with grain yield in the aerobic environment, in total of 20 hydrological environments over a period of five seasons and in five populations in three genetic backgrounds using bulk-segregant analysis (Venuprasad et al. 2011).

## 4. NUTRIENT USE EFFICIENCY

Nitrogen is an imperative element for improving higher grain yield, root development for uptake of water and other nutrient elements from the soil, regulation of flowering time, and grain quality. Several OTLs have been identified for N use efficiency in rice. In a mapping population of Nipponbare and Kasalath, 6-7 OTLs have been identified for glutamine synthase and NADH glutamate synthetase involved in nitrogen uptake pathway (Obara et al. 2001). Similarly, qNUEP-6, pneu9 and QTL for low N tolerance have been reported in rice (Zhou et al. 2017). Over expression of one of the nitrate transporter gene OsNRT3.2b has been reported to increase the yield of rice. Interestingly, a heterotrimeric G protein was identified to regulate N use efficiency in rice (Sun et al. 2014). This gene was previously identified as dense and erect panicle 1 (DEP1) and reported to alter the panicle architecture. In addition, natural variation of DEP1 locus was shown to increase the yield of rice (Huang et al. 2009). Thus, this gene could be used for simultaneously increasing the yield and NUE of rice. The genetic loci associated with N use efficiency were mapped in ASD16 x Basmati 370 population (Senthilvel et al. 2004). In another report, seven QTLs were identified for nitrogen use in the mapping population of IR64 x Azucena (Senthilvel et al. 2008).



Next to nitrogen, phosphorus is considered as one of the major nutrient for rice and essential for better root development (Bovillet al. 2013). The available form of phosphorus in the soil is limited due to its fixation nature in the soil. Several reports have suggested the possibility of improving the genetic potential of rice towards efficient utilization of phosphorus. The major OTL for low phosphorus tolerance 'phosphorus uptake 1' (Pup1) was identified in the aus genotype Kasalath and the causal gene (PSTOL1) was found to be a protein kinase (Gamuyao et al. 2012). It increases the root biomass of rice crop under low P condition. On the other hand, OsPHO1 gene was identified to play an important role in transfer of P from roots to shoots (Secco et al. 2010). Several phosphorus transporter genes have been identified to play an important role in P uptake and remobilization in rice. The expression studies of phosphorus transporter genes have identified PT2 and PT6 gene as high affinity phosphorus transporter gene in rice and *PT8* as gene responsible to transport of P from source to sink organs in rice (Li et al. 2015). Till date, several QTLs have been reported for phosphorus use efficiency (PUE) in rice. Further, Mahender et al. (2017) has done a comprehensive review on QTLs and recent advances on PUE. They reported that to date around 133 P associated QTLs of morpho-physiological traits were available and found to be distributed on all 12 chromosomes and the majority of them were localized on chromosome 1, 2 and 12. A high density SNP mapping of RIL population has identified 26 QTLs for eight PUE traits (Wang et al. 2014). Apart from PUE traits, genetic variation of root traits has been studied to understand the P uptake and low P tolerance in rice (Vejchasarn et al. 2016). Recently, Mehra et al. (2017) characterized purple acid phosphatase (PAP21b) gene from Dular genotype that confers low phosphorus tolerance through enhancing the availability of P present in organic sources in the soil. On the other hand, Yugandhar et al. (2017) screened six N22 mutants in three conditions, normal, low P and AWD and estimated the genetic diversity. They found that *Pup1* gene-specific marker, K-1 was associated with tiller number under low P conditions.

Phosphorus use efficiency can also be improved by mycorrhization of the rice plant with Arbuscular Mycorrhizal Fungi (AMF) that causes significant mobilization of insoluble P in substrate and plant uptake. Arbuscular mycorrhizal fungi inoculated rice through direct or indirect mechanism facilitates uptake of P from poorly soluble P (Panneerselvam et al. 2016a; 2016b), Zn, Cu etc. (Harley and Smith 1983; Wellings et al. 1991). The AMF colonization in roots helps the plants to uptake fixed soil P by rhizosphere modification through multiple mechanisms, by secretion of organic acids, phosphatase enzyme and metabolites like siderophores (Shenoy and Kalagudi 2005; Panneerselvam and Saritha 2017). The mycorrhizal plant also could absorb P from poorly soluble P source like aluminum phosphates, iron and rock phosphate (Shenoy and Kalagudi 2005). Reports suggests that AMF association in plants also causes changes in pH and root exudation profile (Li et al. 2001; Sowarnalisha et al. 2017), which may alter the rhizosphere microbial community and in turn, enhances P solubilization mechanism. However, it was observed that the plant growth performance and nutrient uptake will vary from species to species; hence there is a need for selection of suitable/efficient AMF for better plant growth and development (Bagyaraj et al. 1989).



## 5. RESEARCH ON DIRECT SEEDED RICE AT ICAR-NATIONAL RICE RESEARCH INSTITUTE

The aerobic rice breeding at ICAR-NRRI was initiated with the support of the Asian Development Bank (ADB) by hybridizing high yielding irrigated rice varieties with drought tolerant lines, aerobic rice germplasm and other exotic donors from International Rice Research Institute (IRRI), Philippines. Under this project, large variability of genotypes for the aerobic condition was generated and promising genotypes were selected by adopting the pedigree breeding method. On the other hand, several segregating populations and fixed lines were introduced from IRRI, Philippines, to select superior lines under Cuttack condition. In 2012, a promising variety Apo was identified from AICRIP Varietal Improvement Programme. Apo is one of the popular aerobic variety of Philippines was found suitable in Odisha, and it was released as CR Dhan 200 (CR 2624-IR55423-01; IET 21214) by the Odisha State Sub-Committee on Crop Standards, Notification, and Release of Varieties. At NRRI, promising aerobic lines nominations were started in 2007 to the national AICRIP trials for evaluation of materials at various target locations in different states of the country. Subsequently, nine aerobic varieties were released through CVRC and SVRC (Table 1).

S.No.	Variety	Parentage	Duration (days)	Grain yield (t/ha)
1	CR Dhan 200 (Pyari)	CR 2624-IR 55423-01	115-120	4.0
2	CR Dhan 201	IRRI 76569-259-1-2-1/ CT 6510-24-1-2	110-115	3.8
3	CR Dhan 202	IRRI 148/IR 78877-208-B-1-1	110	3.7
4	CR Dhan 203 (Sachala)	IR78877-208-B-1-1/ IRRI 132	110-115	4.0
5	CR Dhan 205	N22/Swarna	105-110	4.2
6	CR Dhan 206	Brahmannaki/ NDR 9930077	105-110	4.2
7	CR Dhan 207 (Srimati)	IR71700-247-1-1-2/ IR57514-PMI 5-B-1-2	110 -115	4.5
8	CR Dhan 209 (Priya)	IR72022-46-2-3-3-2/IRRI 105	110 -115	4.5

Table 1. Rice varieties released from ICAR-NRRI for dry direct/aerobic condition.

Upland rice is known to have significant uptake ability of P under deficient condition. Therefore, 70 upland rice genotypes and wild species (*O.nivara* and *O. rufipogon*) were screened under P deficient soil (6 ppm) with two tolerant checks (Kasalath and Dular) and one susceptible check (IR 36). Among them, eight upland genotypes and four wild species exhibited superiority over the positive checks. They were genotyped to assess the presence of *Pup1* allele. Genotypes AC100062, AC100117, Dular, Sekri, Brown gora and AC 100117 had similar amplification pattern with Kasalath (NRRI annual report 2014-15). Similarly, Elssa Pandit et al. (2016) genotyped 96 upland cultivars and landraces for better P-uptake. Among them, 46 possessed the Pup1 locus that accounts for 47.92% of the total genotypes considered. The genotypes N22, Dinoroda, Bowde, Bamawpyan, Tepiboro, Karni, Lalsankari, Surjamukhi, Hazaridhan and Kalinga III were positive for two closest flanking and two gene specific *Pup1* markers.

81



## 6. KNOWLEDGE GAPS

Direct seeded rice is a viable alternative to TPR and suitable for targeted areas suffering from water availability and labor scarcity. Further, DSR/aerobic rice is found most suitable for rainfed lowland areas where insufficient precipitation occurs, delta regions where there is no availability of water in-time or delay in water release from reservoir (Anandan et al. 2015), limited water during early stage of crop growth but later crop faces flood, pumping from deep bore well and favorable upland has access to supplementary irrigation (Bouman 2001) are the most suitable location to adapt DSR/aerobic rice. Accordingly, Chhattisgarh, parts of Bihar, Jharkhand, Karnataka, Odisha, Tamil Nadu, and eastern Uttar Pradesh are the projected areas, where there is an uneven distribution of rainfall and frequent occurrence of soil moisture limitation. Further, the maintenance of physical soil structure in DSR helps to have timely sowing of succeeding crop.

The success of DSR system depends on availability of suitable variety and crop management practices. However, non-availability of suitable breeding program and limited knowledge on the genetics of donors are the major drawback of this system to achieve adaptable and maximum yield potential under DSR. The ICAR-NRRI, Cuttack has released several rice varieties (Table 1) suitable for DSR/aerobic condition and the grain yield of those genotypes yield 4.5t/ha (Anandan et al. 2015) under continuous aerobic condition. To improve the grain yield further, several traits adaptable for DSR need to be addressed.

The phenotypic traits directly relevant to DSR genotypes should have the increased ability to germinate from deeper soil depth with longer coleoptile and mesocotyl length and uniform germination ability to have uniform plant population with early seedling vigour (Anandan et al. 2016a; 2016b). As weed has the major impact on DSR, uniform plant population with uniform height facilitates timely intercultural operation to manage weeds. Increased numbers of nodal roots, root length density, lateral root length and branching with more root hairs for proper nutrient uptake under dry conditions are necessary traits for DSR (Fig. 2) (Kumar et al. 2017). Anaerobic germination is another important trait that improves crop establishment in uneven field or flash flood after rain. Roots of DSR variety should have sufficient plasticity to function under both aerobic and water logging condition. During early stage of crop establishment, the seedling should possess early vigour with good canopy cover to smoother the weed growth. The later stage of crop should have low specific leaf area, high chlorophyll, medium plant height (110-120 cm), early flowering, slim and sturdy culm, 250-300 panicles per meter square, more number of filled grains per meter square, retaining panicle height within the canopy level, erect boot leaf and high harvest index (Fig. 2). Deep rooting plants would be more advantageous, as they could mine water from deeper layer (Fig. 1). On the other hand, switching over the rice crop from flooded condition to aerobic/dry rice, soil can reduce the indigenous supply of P, Fe (Fan et al. 2012) and Zn. Continues cultivation of rice over the years under dry condition, would result in soil sickness. Moreover, soil Fe fertilization increased concentration in shoot and dry weight at tillering, but



failed to increase the same during physiological maturity (Fan et al. 2012). Therefore, the traits starting from germination to maturity needs to be addressed to achieve yield on par with TPR.

## 7. RESEARCH AND DEVELOPMENT NEEDS

Under the growing water scarcity in Indian agriculture scenario, rice cultivation is gradually progressing from continuous flooded TPR system to partial or complete aerobic condition. Therefore, a new plant system that differs from regular transplanted rice has to be developed for this condition (Fig. 2). The varieties needed for DSR should have wider adaptation to suit the aerobic rice ecosystem with maximum water use efficiency, along with less yield penalty.

#### 7.1. Breeding for yield component traits

Grain yield is the collective phenotypic expression of yield component traits whereas, adaptability is due to buffering capacity of genotypes for which many minor genes are in additive response. Thus, the accrual of favourable genes, those expressing at critical stage of rice plant under DSR/aerobic condition has to be combined.

#### 7.2. Seedling vigour and its associated traits for early establishment

Early and uniform crop establishment is necessity for DSR. High seedling vigour, improved field emergence, weed competitiveness, tolerance against anaerobic condition and reduced moisture stress during germination (Mahender et al. 2015), enhanced foliar growth to combat weeds at the vegetative stage (Dingkuhn et al. 1991), seedling dry weight, rapid shoot growth, shoot dry weight, mesocotyl and coleoptile length (Fujino et al. 2008; Trachsel et al. 2010), germination rate, germination index, amylase activity, root activity and chlorophyll content (Diwan et al. 2013; Dang et al. 2014; Sandhu et al. 2015) are significantly contributes to early establishment in direct seeded rice. Additionally, these traits would facilitate to overcome limited moisture condition and/or submergence during monsoon time. Therefore, genetic improvements in rice breeding program for direct seeded condition should focus on improving those traits to make rice suitable for DSR. In recent years, several QTLs were identified for early establishment of rice seedling and they were elaborated well by Mahender et al. (2015) and Kumar et al. (2017). QTLs for traits related to early seedling vigour such as shoot length, germination rate and root dry weight in 28 days old seedling (Haritha et al. 2017; Anandan et al. 2016a; Mahender et al. 2015) and gEMM1.1 for early uniform emergence were identified form direct seeded condition (Dixit et al. 2015). Successful introgression of these QTLs would provide an opportunity to the early establishment and increases the plant population, thereby smothers the weed growth. Early seedling vigour cultivars are reported to be a lowcost, durable and decrease the detrimental effect of herbicide on the environment (Anandan et al. 2016a).



### 7.3. Understanding the traits for vegetative and reproductive phases

The photosynthates accumulated during vegetative stage from available biomass of aerial and underground portion determines the final output of the crop. Therefore, moderate tillering of 10-12 numbers and enhanced assimilate export from leaves to stems during the late vegetative and reproductive phases are important to achieve higher grain yield by grain filling (Dingkuhn et al. 1991). Ghosh et al. (2012) from the ICAR-NRRI, Cuttack has observed that 17% yield decline under aerobic system occur due to inhibition in the structural development of roots as increase in the concentration of hydrogen peroxide (24.6%) and proline (20%) and a lower concentration of total soluble protein (20%). Therefore, the OTLs identified under DSR would be more appropriate to utilize in rice breeding for DSR than the OTLs identified form TPR condition. Introgression of such QTLs would definitely be beneficial and improves better adaptation to DSR condition with high yield potential. Successful introgression of such OTLs provides an opportunity to study their performance and interaction between them. Sandhu et al. (2015) identified QTLs for nodal root (qNR4.1 and qNR5.1) and root hair density (gRHD1.1 and RHD5.1) under direct seeded condition. Similarly, Dixit et al. (2015) reported OTLs qLDG3.1 and qLDG4.1 for lodging tolerance. Several grain yield QTLs were identified (qGY1.1, qGY2.1, qGY2.2, qGY8.1 and qGY10.1) by Sandhu et al. (2013; 2015) under aerobic condition. Therefore, the breeding program for DSR should aim to increase the yield and adaptability by introgressing such QTLs for the benefit of farmers in the target areas struggling for water and labor.

#### 7.4. Breeding for biotic stresses

Rice cultivation under aerobic favors blast (*Magnaporthe grisea*), bacterial leaf blight (BB) (*Xanthomonas oryzae* pv. oryzae) and brown spot (*Helminthosporium oryzae*). Therefore, rice varieties of aerobic should have durable resistance genes for blast and BB. This could also help to reduce resources to be incurred and environmental pollution. Brown spot often occurs in poorly managed and deficient soils under inadequate soil moisture. Therefore, balanced dose of nitrogenous fertilizer needs to be maintained with sufficient soil moisture to avoid the incidence of blast and brown spot. Stem borer and leaf folder are the major concern of aerobic rice. Cultivation of resistance varieties and summer ploughing are recommended to reduce pest incidence in aerobic. The menace of root-knot nematode *Meloidogyne graminicola* is increasing under aerobic and causes severe damage to rice plants. Therefore, raising resistance varieties are recommended to avoid yield reduction.

#### 7.5. Breeding for nutrient use efficiency

Saturation level increases the availability of nutrients P and Fe. There are reports on soil sickness, which appears after few years of aerobic rice cultivation. Moisture at field capacity in aerobic condition may reduce the nutrient availability. Therefore, detailed study must be taken to understand the nutrient dynamics of major and micronutrient and their bioavailability. Pal et al. (2007) observed the differential responses of rice cultivars to applied Fe. In an experiment to test rice genotypes under aerobic condition for Fe, two lines (CT 6510-24-1-2 and IR 71525-19-1-1)



performed better compared to the varieties IR 36 and IR 64 suitable for TPR. These deferential responses sound that inherent ability of the genotype plays important role in Fe absorption. Sandhu et al. (2015) has reported OTLs gN5.1, gP5.2, gFe5.2 for nodal root and root hair density, that plays major role in higher nutrient uptake (Al, Fe, and P), under DSR. OTLs for higher nutrient uptake, higher nutrient concentration and root hair density were also found to be co-localized in the same region. Enhancing root growth with root hair density enhances P uptake (Mahender et al. 2017). Fine mapping of *Pup1* region lead to the identification of P starvation tolerance 1 (PSTOL1) gene encoding a PSI protein kinase. Under P deficient condition, PSTOL1 enhances the early root elongation and improves the grain yield (Gamuyao et al. 2012). Recently Mehra et al. (2017) characterized purple acid phosphatase (PAP21b) gene from Dular that enhances the uptake of P present in organic form in the soil. Thus, stacking of PSTOL1, PAP21b, and OTLs of nodal root and root hair density into elite varieties by marker-assisted selection would definitely improve nutrient concentration in plants under aerobic condition. Stacking up of nutrient uptake genes/OTLs may result in improved performance by complementary effects will reduce the cost incurred by resource-poor farmers.

## 7.6. Molecular understanding and their role of microbes to improve nutrient use efficiency

The genomics and transcriptomics studies revealed that a successful AMF root colonization is controlled by the genotype of plants. The HAR1 gene i.e. Hypernodulation and Aberrant Root Formation1 are reported to control number of root nodules in legumes and the same gene also plays a role in the regulation of AMF symbiosis (Solaiman et al. 2000), but the mode of action of this gene is still not understood. The genetic requirements for rhizobial and AMF association in plants overlap in a common symbiosis pathway (CSP), which leads to successful root nodule and AMF symbiosis (Tirichine et al. 2006). The CSP plays an important role in accommodating root nodule and AMF symbionts, by which plant cells vigorously decompose their cell wall structures to facilitate microbial colonization (Parniske, 2000). The effects of the Oryza sativa calcium/calmodulin-dependent protein kinase (OsCCaMK) indicated that a strong expression of OsCCaMK was detected in rice roots, where mycorrhizal colonization is expected to occur (Parniske 2009). The other study revealed that OsCCaMK gene expression has the positive correlation with the diversity of root-associated bacteria and the growth of rice plants (Ikeda et al. 2011). The above information indicates that studying the plant associated gene and its expression related to the specific microbial association is very important to develop rice varieties, which have affinity towards the specific group of microbes to improve the NUE in rice (Fig. 2).

## 8. WAY FORWARD

The modern plant breeding has moved from classical breeding to precision breeding by adapting powerful indirect selection technique of employing molecular

85



marker technology. During the last two decades, the modern plant breeding is progressing in faster pace; more number of popular rice varieties of favorable land to marginal land with assured yield is developed by researchers in India. Several genes were identified and characterized for their functions related to yield, nutrient deficiency tolerance, biotic and abiotic stresses. Therefore, designing of a plant variety with desired traits becomes possible for any specific ecosystem. However, more efforts are needed to identify new genes responsible for nutrient deficiency tolerance and water use efficiency. On the other hand, further understanding is required to know the genes involved for regulating the differences in use efficiency and tolerance mechanism. This would maximize their role in crop improvement program for resource use efficiency. Developing next-generation rice suitable for the direct seeded system is very much necessary to tackle increased food demand under the limited labor, land, water, and nutrient during this changing climate period. The molecular approaches along with best crop management could significantly increase the productivity in aerobic rice cultivation.

#### References

- Ali J, Xu JL, Gao YM, Fontanilla MA, Li and ZK (2012) Green super rice (GSR)technology: An innovative breeding strategy- achievements & advances. In: proceedings of The 12<sup>th</sup> SABRAO congress-Plant Breeding towards 2025: Challenges in a rapidly changing world Chiang Mai, Thailand, 13-16th January, 2012 p16-17.
- Anandan A, Anumalla M, Pradhan SK and Ali J (2016a) Population Structure, Diversity and Trait Association Analysis in Rice (*Oryza sativa* L.) Germplasm for Early Seedling Vigor (ESV) Using Trait Linked SSR Markers. PLoS ONE 11(3): e0152406. doi:10.1371/ journal.pone.0152406
- Anandan A, Mahender A, Rupa Kumari, Pradhan SK, Subudhi HN, Thirugnanakumar S and Singh ON (2016b) Appraisal of genetic diversity and population structure in assorted rice genotypes for early seedling vigour trait linked markers. Oryza 53(2): 113-125.
- Anandan A, Pradhan SK and Singh ON (2015) Direct seeded rice: an approach to trim down water consumption and labour. http://www.krishisewa.com/cms/articles/production-technology/ 578-direct-seeded-rice.html
- Anandan A, Pradhan SK and Singh ON (2015) A system of rice cultivation for water shortfall irrigated and lowland areas: Aerobic rice an overview. Popular Kheti 3(3):8-13.
- Atlin GN, Lafitte HR, Tao D, Laza M, Amante M and Courtois B (2006) Developing rice cultivars for high-fertility upland systems in the Asian tropics. Field Crops Research 97:43-52.
- Banba M, Gutjahr C, Miyao A, Hirochika H, Paszkowski U, Kouchi H and Imaizumi-Anraku H (2008) Divergence of evolutionary ways among common sym genes: CASTOR and CCaMK show functional conservation between two symbiosis systems and constitute the root of a common signaling pathway. Plant and cell physiology 49(11):1659-71.
- Bouman BAM, Peng S, Castaneda AR and Visperas RM (2005) Yield and water use of irrigated tropical aerobic rice systems. Agricultural Water Management 74:87–105.
- Bovill WD, Huang CY and McDonald GK (2013) Genetic approaches to enhancing phosphorususe efficiency (PUE) in crops: challenges and directions. Crop and Pasture Science 64(3):179-198.
- Breidenbach B, Pump J and Dumont MG (2016) Microbial community structure in the rhizosphere of rice plants. Frontiers in microbiology 6:1537.



- Brune A, Frenzel P *and* Cypionka H (2000) Life at the oxic–anoxic interface: microbial activities and adaptations. FEMS Microbiology Reviews 5:691-710.
- Dick SM and MW Rosegrant MW (2001) Overview, in *Overcoming Water Scarcity and Quality Constraints*.International Food Policy Research Institute, Washington, DC.
- Dixit S, Grondin A, Lee CR, Henry A, Olds TM and Kumar A (2015) Understanding riceadaptation to varying agro-ecosystems: trait interactions and quantitative trait loci. BMC Genomics 16 (1):86.
- Elssa Pandit, Sahoo A, Panda RK, Mohanty DP, Pani DR, Anandan A and Pradhan SK (2016). Survey of rice cultivars and landraces of upland ecology for *Phosphorous uptake 1 (Pup1)* QTL using linked and gene specific molecular markers. Oryza 53(1):1-9.
- FAI (2000-2007) Fertilizer statistics. Fertilizer Association of India, New Delhi
- Fan X, Karim Md K, Chen X, Zhang Y, Gao X, Zhang F and Zou C (2012) Growth and Iron Uptake of Lowland and Aerobic Rice Genotypes under Flooded and Aerobic Cultivation, Communications in Soil Science and Plant Analysis, 43(13):1811-1822.
- Gamuyao R, Chin JH, Pariasca-Tanaka J, Pesaresi P, Catausan S, Dalid C and Heuer S (2012) The protein kinase *Pstol1* from traditional rice confers tolerance of phosphorus deficiency. Nature 488(7412):535.
- Garg NK and Hassan Q (2007) Alarming scarcity of water in India. Current Science 93(7): 932-941.
- George T, Magbanua R, Roder W, Vankeer K, Trebuil G and Reoma V (2001) Upland rice response to phosphorus fertilization in Asia. Agronomy Journal 93:1362-1370.
- Guang-hui X, Jun Y, Hua-qi W and Bouman BAM (2008) Progress and Yield Bottleneck of Aerobic Rice in the North China Plain: A Case Study of Varieties Handao 297 and Handao 502. Agricultural Sciences in China 7:641-646.
- Pathak H, Mohanty S, Jain N and Bhatia A (2010) Nitrogen, phosphorus, and potassium budgets in Indian agriculture. Nutrient Cycling in Agroecosystems 86:287–299.
- Hardoim PR, Andreote FD, Reinhold-Hurek B, Sessitsch A, Van Overbeek LS and Van Elsas JD (2011) Rice root-associated bacteria: insights into community structures across 10 cultivars. FEMS Microbiol Ecology 77:154–164.
- Haritha G, Swamy BPM, Naik ML, Jyothi B, Divya B and Sarla N (2018) Yield Traits and Associated Marker Segregation in Elite Introgression Lines Derived from O. sativa  $\times$  O. nivara. Rice Science 25(1):19-31.
- Harley JL and Smith SE (1983) Mycorrhizal symbiosis. Academic Press London
- Huang X, Qian Q, Liu Z, Sun H, He S, Luo D *and* Fu X (2009) Natural variation at the DEP1 locus enhances grain yield in rice. Nature genetics 41(4):494-497.
- Ikeda S, Okubo T, Anda M, Nakashita H, Yasuda M, Sato S, Kaneko T, Tabata S, Eda S, Momiyama A and Terasawa K (2010) Community-and genome-based views of plant-associated bacteria: plant-bacterial interactions in soybean and rice. Plant and Cell Physiology 51(9):1398-410.
- Ikeda S, Okubo T, Takeda N, Banba M, Sasaki K, Imaizumi-Anraku H, Fujihara S, Ohwaki Y, Ohshima K, Fukuta Y and Eda S (2011) The genotype of the calcium/calmodulin-dependent protein kinase gene (CCaMK) determines bacterial community diversity in rice roots under paddy and upland field conditions. Applied and environmental microbiology 77(13):4399-405.
- Kumar V and Ladha JK (2011) Direct seeding of rice: recent developments and future research needs. Advances in Agronomy 111:297–413.



- Li XL and Christie P (2001) Changes in soil solution Zn and pH and uptake of Zn by arbuscular mycorrhizal red clover in Zn-contaminated soil. Chemosphere 42:201-207.
- Mahender A, AnandanA and Pradhan SK (2015) Early seedling vigour, an imperative trait for direct-seeded rice: an overview on physio-morphological parameters and molecular markers. Planta 241:1027–1050.
- Mahender A, Anandan A, Pradhan SK and Singh ON (2017) Traits-related QTLs and genes and their potential applications in rice improvement under low phosphorus condition, Archives of Agronomy and Soil Science DOI: 10.1080/03650340.2017.1373764.
- Obara M, Kajiura M, Fukuta Y, Yano M, Hayashi M, Yamaya T *and* Sato T (2001) Mapping of QTLs associated with cytosolic glutamine synthetase and NADH- glutamate synthase in rice (*Oryza sativa* L.). Journal of Experimental Botany 52:1209–1217.
- Pal S, Datta SP, Rattan RK *and* Singh AK (2008) Diagnosis and Amelioration of Iron Deficiency under Aerobic Rice. Journal of Plant Nutrition 31(5):919-940.
- Panneerselvam P, Anushree PM, Kumar U, Anandan A, Parameswaran C, Anjani Kumar and Nayak AK (2016a) Studies on host preference of AM fungi in different aerobic rice varieties under elevated carbon dioxide condition. In: 57<sup>th</sup> Annual conference of association of Microbiologist of India and International symposium on Microbes and Biosphere: what's New What's next, November 24-27, 2016, Guwahati University, Assam, India.
- Panneerselvam P, Kumar U, Saha S, Adak T and Munda S (2016b) Effect of Bis-pyribac sodium on Arbuscular Mycorrhizal fungal association in rice. NRRI Newsletter 37 (1):18.
- Panneerselvam P and Saritha B (2017) Influence of AM fungi and its associated bacteria on growth promotion and nutrient acquisition in grafted sapota seedling production. Journal of Applied and Natural Science 9(1):621-625.
- Parniske M (2000) Intracellular accommodation of microbes by plants: a common developmental program for symbiosis and disease?. Current opinion in plant biology 3(4):320-8.
- Sandhu N, Jain S, Kumar A, Mehla BS and Jain R (2013) Genetic variation, linkage mapping of QTL and correlation studies for yield, root, and agronomic traits for aerobic adaptation. BMC Genetics 14:104–119.
- Sandhu N, Torres RO, Cruz MTS, Maturan PC, Jain R, Kumar A and Henry A (2015) Traits and QTLs for development of dry direct-seeded rainfed rice varieties. Journal of Experimental Botany 66(1):225–244.
- Schindler DW, Carpenter SR, Chapra SC, Hecky RE and Orihel DM (2016) Reducing phosphorus to curb lake eutrophication is a success. Environmental Science & Technology. 50:8923"8929.
- Secco D, Baumann A and Poirier Y (2010) Characterization of the rice PHO1 gene family reveals a key role for OsPHO1; 2 in phosphate homeostasis and the evolution of a distinct clade in dicotyledons. Plant physiology 152(3):1693-1704.
- Senthilvel S, Govindaraj P, Arumugachamy S, Latha R, Malarvizhi P, Gopalan A and Maheswaran M (2004) Mapping genetic loci associated with nitrogen use efficiency in rice (*Oryza* sativa L.). In: New directions for a diverse planet: Proceedings of the 4<sup>th</sup> International Crop Science Congress.
- Senthilvel S, Vinod KK, Malarvizhi P and Maheswaran M (2008) QTL and QTL× environment effects on agronomic and nitrogen acquisition traits in rice. Journal of integrative plant biology 50(9):1108-1117.
- Shashidhar HE (2012) An eco-friendly aerobic rice BI 33 (Anagha). Available at www.aerobicrice.in.
- Shenoy VV and Kalagudi GM (2005) Enhancing plant phosphorus use efficiency for sustainable cropping. Biotechnological Advances 23:501-513.



- Sowarnalisha S, Panneerselvam P, Tapas Chowdhury, Anjani Kumar, Upendra Kumar, Afrin Jahan, Ansuman Senapati and Anandan A (2017) Understanding the AM fungal association in flooded rice under elevated CO2 condition. Oryza 54 (3):290-297.
- Sun H, Qian Q, Wu K, Luo J, Wang S, Zhang C and Han R (2014) Heterotrimeric G proteins regulate nitrogen-use efficiency in rice. Nature Genetics 46(6):652-656.
- Tirichine L, Imaizumi-Anraku H, Yoshida S, Murakami Y, Madsen LH, Miwa H, Nakagawa T, Sandal N, Albrektsen AS, Kawaguchi M, Downie A (2006) Deregulation of a Ca2+/calmodulindependent kinase leads to spontaneous nodule development. Nature 441(7097):1153-6.
- Somanathan E (2010) Taming the Anarchy: Groundwater Governance in South Asia. Indian Growth and Development Review 3(1):92-94.
- Vejchasarn P, Lynch JP and Brown KM (2016) Genetic variability in phosphorus responses of rice root phenotypes. Rice 9(1):1-16.
- Venuprasad R, Bool ME, Dalid CO, Bernier J, Kumar A and Atlin GN (2009) Genetic loci responding to two cycles of divergent selection for grain yield under drought stress in a rice breeding population. Euphytica 167:261-269.
- Venuprasad R, Bool ME, Quiatchon L, Sta Cruz MT, Amante M and Atlin GN (2012). A large effect QTL for rice grain yield under upland drought stress on chromosome 1. Molecular Breeding 30: 535–547.
- Vinod KK and Heuer S (2012) Approaches towards nitrogen- and phosphorus-efficient rice. AoB Plants p: pls028. http://doi.org/10.1093/aobpla/pls028
- Visperas RM, Peng S, Bouman BAM and Castaneda AR (2002) Physiological analysis of yield gap between flooded and aerobic rice. Philippine Journal of Crop Science27: 50.
- Wang K, Cui K, Liu G, Xie W, Yu H, Pan J and Peng S (2014) Identification of quantitative trait loci for phosphorus use efficiency traits in rice using a high density SNP map. BMC genetics 15(1):155.
- Wellings NP, Wearing AH and Thompson JP (1991) Vesicular-arbuscular mycorrhizae (VAM) improve phosphorus and zinc nutrition and growth of pigeon pea in a Vertisol. Australian Journal of Agricultural Research 42:835–845
- Wissuwa M and Ae N (2001) Genotypic variation for tolerance to phosphorus deficiency in rice and the potential for its exploitation in rice improvement. Plant Breeding 120:.43–48.
- Ye C, Argayoso MA, Redona ED, Sierra SN, Laza MA, Dilla CJ, Mo Y, Thomson MJ, Chin J, Delavina CB, Diaz GQ *and* Hernandez JE (2012) Mapping QTL for heat tolerance at flowering stage in rice using SNP markers. Plant Breeding 131:.33-41.
- Yugandhar P, Basava RK, Desiraju S, Voleti SR, Sharma RP and Sarla N (2017) Identifying markers associated with yield traits in Nagina22 rice mutants grown in low phosphorus field or in alternate wet/dry conditions. Australian Journal of Crop Science 11(05):548-556.
- Zhou Y, Tao Y, Tang D, Wang J, Zhong J, Wang Y and Liang G (2017) Identification of QTL Associated with Nitrogen Uptake and Nitrogen Use Efficiency Using High Throughput Genotyped CSSLs in Rice (Oryza sativa L.) Frontiers in Plant Science 8.
- Zhu Z and Chen D (2002) Nitrogen fertilizer use in China-contributions to food production, impacts on the environment and best management strategies. Nutrient Cycling in Agro ecosystems 63:117-27.\*



## Genetic Improvement of Rice for Aroma, Nutrition and Grain Quality

### S Sarkar, SSC Pattanaik, K Chattopadhyay, M Chakraborti, P Sanghamitra, N Basak, A Anandan, S Samantaray, HN Subudhi, J Meher, MK Kar, B Mondal and AK Mukherjee

## SUMMARY

Rice is the staple food for more than half of the world population satisfying the variable quality preferences of consumers across the globe. Therefore, there is no universal quality for rice. Besides quantitative nature of inheritance, effect of environment in expression, destructive methods of estimation and high error rates in analysis, complicates breeding for quality traits. The known traits are often unable to explain the end use quality variations. Non destructive trait descriptors that explain variations in quality need to be identified. These parameters can then be used to formulate regression models to predict end-use quality. Large number of QTLs and genes controlling quality has been reported. However, their functionality across genotypes needs validation for their effective utilization in breeding programmes. An in-depth understanding of the underlying mechanisms controlling grain quality will enable breeders to precisely connect the missing links with greater efficiency. The present chapter will attempt to provide an overview of the scientific and technological advances in rice grain quality research, with special emphasis to genetic studies and breeding efforts in India and abroad.

#### **1. INTRODUCTION**

Quality in rice refers to its physical and physico-chemical properties before and after cooking. Physical properties include the physical appearance of grain, its size, shape, color, uniformity in overall appearance, luster etc. Specific density of grain, hulling and milling recovery, head rice recovery etc. represents Millers' qualities. Physicochemical properties include the biochemical characters of the grain especially amylose, amylopectin, soluble starch, resistant starch, protein and micronutrient content besides gel consistency, gelatinization temperature, pasting properties, water uptake, volume expansion ratio, amylographic properties, etc. A new dimension to rice quality has been added in the form of its sensory qualities which include taste, flavor, uniformity of particles and mouth feel after chewing of the cooked whole grain etc. The sensory qualities are highly subjective in nature and vary widely from person to person. Among all other sensory qualities, aroma needs special mention due to its high market demand. Basmati rice is one such example where the premium cooking and eating quality along with pleasant aroma created a multi-billion dollar export industry to India. However, preference for aroma is highly location specific. Besides basmati there are other categories of aromatic rice, which fetch better prices in the



market. Looking into the wide acceptability of rice as staple, cuisines and desserts, the quality of this miracle grain is divided into four broad categories physical appearance, milling properties, cooking & eating characteristics and nutritional and nutraceutical quality. Nutritional quality includes grain protein, vitamins and micronutrient content while nutraceutical properties deal with the healing compounds. Individually each component plays significant role in maintaining good health.

Quality preferences in rice are highly variable. Different countries have different requirements for quality. Even within countries, an array of preferences can be observed. Therefore specific quality profiles need to be developed through extensive survey among consumers, millers and farmers in the target area of breeding. A preliminary survey on quality preferences of urban populations of eastern and southern India have been undertaken by Social scientists at IRRI. They concluded that medium slender grain type is preferred over long slender among both the populations. Aroma was priority for 37% respondents of eastern India. The preference for aroma was lower among the respondents of South India. A huge variation in grain type preference was observed among different cities of Eastern India revealing highly variable consumer preference for quality traits in the region. Appearance of grain, its cooking and eating qualities are highly significant in deciding the market value of produce. No rice is consumed if its cooking and eating quality does not match the preference of consumers. Besides the cooking quality of whole grain, rice processing quality i.e. ability of rice to be processed to different end products like puffed rice, popped rice, flattened rice etc., is gaining attention among millers and consumers. All these dimensions of rice quality should be understood well before initiating breeding efforts to improve it.

More over, the quality traits are quantitative in nature are quality traits and are highly influenced by environment. The existence of multiple genes and epistatic interactions among them complicates their breeding procedure. The situation is further complicated by the variable reports on genetic basis of these traits in different populations alongwith destructive nature of their phenotyping. However, with the advancements in laboratory methods and instrumentation, techniques for precise phenotyping of these traits are becoming available. Current advancements in the science of plant genomics will help the breeders to relate the quality traits to specific genes or genomic regions which are expected to transform the breeding outlook of quality improvement in near future.

In the present chapter, we have attempted to depict the shift of focus on rice quality in the time frame of last seventy years. How the research dimensions changed with respect to quality, making it integral criteria in rice genetic improvement in today's world? Multidimensional aspects of rice grain quality have been discussed especially with respect to their genetic and molecular mechanisms. The underlying genes for various quality traits, their interactions and the resources available for precise breeding of these traits have been discussed in details, besides the significant achievements through classical breeding approaches. In Fig. 1, an overview of all the components of rice quality and their importance in varietal improvement has been presented graphically.

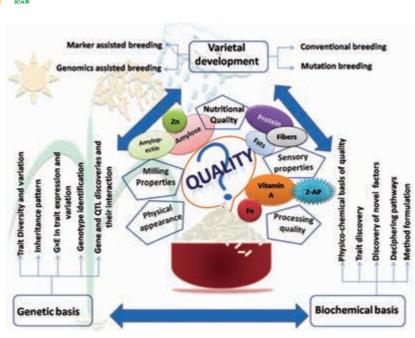


Fig.1. Graphical representation of the chapter

## 2. STATUS OF RESEARCH IN RICE QUALITY

Several studies have been undertaken to understand the genetic basis of quality, and ways to improve it through established principles of genetics. Before moving into the detailed discussion on genetic improvement of quality traits, it is essential to remember that genetic studies can be undertaken accurately only when precise methods of trait phenotyping do exist. Since grain quality traits are mostly controlled by biochemical components present in the grain, knowledge of biochemistry is important to understand the variation in these traits. Initial studies therefore focused on investigating the physico-chemical basis of different quality traits and formulating methods to quantify them. Work of Juliano at IRRI, Philippines and Bhattacharya at CFTRI, Mysore, India needs special mention. The two researchers contributed immensely to the understanding of physicochemical basis of rice quality during the early days of 1960's and 70's and throughout. Geneticists have utilized the advancements made in phenotyping of these quality traits for deciphering their genetic basis. Significant amount of information is now available with the breeders for pecise improvement of quality traits.

#### 2.1. Phenotyping for quality

Among the various grain quality factors, aroma is one of the most easily recognizable sensory qualities which have been strongly selected by cultivators over generations. Chewing test has long been used for selection of aroma with the subsequent development of KOH test followed by development of more sophisticated



and sensitive instruments like gas chromatography mass spectrometry (GCMS) to precisely measure aroma and detect the volatile compounds responsible for it. Despite of the unavailability of standard measures for the sensory attributes including aroma, taste and flavor of cooked rice; the traits have been selected by the farmers over generations which is well revealed by their presence in local varieties and landraces.

There are several parameters to estimate the cooking and eating quality of rice. Amylose content, gel consistency, gelatinization temperature and pasting properties are objective parameters deciding the cooking and eating quality and have long been being recognized and worked upon by researchers. Sensory qualities like aroma, flavor and mouth feel are subjective parameters and are more recently recognized as important in deciding the market value of the produce. The traits have been preserved by farmers in form of landraces and local varieties at small scales for their own consumption. Sensory qualities largely depend on human perception and are therefore very complex to measure. Hence, the support of social scientists, psychologists and neurologists is also envisaged in formulating methods for precise measurement of these traits. Research efforts have been undertaken for identification of component traits which together decide the ultimate consumers' preference. The component traits are then subjected to scoring by trained panelists. However, such studies were realized to be loaded with human errors, as perception varies with health, environment, social and psychological status of the scorer. In order to overcome the variable human errors in these studies, instruments such as the texture analyzer, colorimeter, luster analyzer, taste meter, electronic tongue and electric nose have been designed to imitate human perception. Multiple regression equations based on the original data from human perceived scores and instrument perception were developed to provide indirect methods of measuring the sensory traits. The science of trait identification for better explanation of sensory qualities is in its nascent stage of evolution and needs further refinements. Methods to measure the nutritional quality of rice grain viz. protein, iron, zinc and other micronutrients evolved later with the advancement in instrumentation during twentieth century. Grain protein content is measured by the basic method of Kjeldahl while micro-nutrients are estimated by their basic property of emitting a particular wavelength of light when moved back to their ground state after ionization. Nondestructive methods of trait estimation have been formulated utilizing the principle of spectrophotometry and calibrating the same using multiple regression equations.

Milling qualities are comparatively easy to measure as they are highly correlated with the physical attributes of grain and are very important for the acceptance of variety in the market. Chalkiness has been found to significantly affect the milling qualities by disturbing the packing of starch granules in the grain. In the chalky area, the starch granules are loosely bound reducing its strength to endure the processing during hulling and milling. The grain breaks in the chalky area reducing the head rice recovery. Studies under Scanning electron microscope could correlate the orientation of starch molecules with chalkiness and milling recoveries. Table 1 represents the quality traits and tests available for their estimation.



Trait	Method
Length, breadth, width, etc	Vernier Callipers, Digital Annadarpan Image Analyzer
Moisture	Moisture meter
Cooking quality	Laboratory based methods viz. Alkali spreading value, KOH test
Pasting properties	Rheovisco analyzer (RVA)
Protein content	Kjeldhal method, Near Infra Red Spectrophotometer
Antioxidants	Spectrophotometry, HPLC
Micronutrient content	Atomic Absorption spectrophotometer (AAS),XRF
Appearance	Gloss meter, "Mido" meter, "Hunter Lab" Colorimeter
Aroma	KOH test, GC, GCMS, Electronic nose
Taste	HPLC, Enzyme kit, Taste sensor, Taste analyzer, Electronic tongue
Texture	Texturometer, Texture analyzer, Tensipresser

#### Table 1. Quality traits and their methods of evaluation.

#### 2.2. Major biochemical components of rice quality

For a comprehensive understanding of quality aspects of rice grains, it is very important to know about their biochemical bases. The major constituent of rice endosperm is starch which comprises about 90% of the total dry weight of polished grains. This is followed by protein, fats, fibers, vitamins and micronutrients. Comprising the bulk of the grain, starch quantity and quality play leading role in deciding almost all quality traits. Amylose and amylopectin together constitute the starch molecule and their relative abundance decides the starch quality. Amylose is long chained polysaccharide with less branching while amylopectin is highly branched. Higher content of amylose makes the rice fluffier, dry and non-sticky with better grain separation of grains after cooking. However, beyond a certain limit (when amylose is >25%) it makes the rice hard when kept for longer time after cooking. On the other hand, lower values of amylose (<15%) or its absence makes the rice sticky and glutinous (waxy rice). However, deviations to this correlation do exist. These deviations have been explained on the basis of amylose-amylopectin ratio or degree of branching of these two chains. Besides cooking quality, starch is vital in deciding the eating and product processing quality in rice. Nature of binding of starch molecules during grain filling decides the overall appearance of raw grain.

The second major component of rice endosperm is protein (vary from 5-12% of grain weight), which is lowest among cereals. However rice protein is best in digestibility among cereal proteins due to the higher content of (~80%) glutelins. Rice protein is also most balanced among cereal proteins due to higher content of lysine and tryptophan, the limiting essential amino acids in cereals. In addition to the traditional quality features, the focus of developing rice as nutricereal has gained momentum in last two decades. Considering rice as staple food for billions, this shift in focus from food security to nutritional security bears great significance. Fats are important in the bran layer of rice as they are abundant there and are used to extract



high quality  $\gamma$ -oryzionol (rice bran oil) containing  $\omega$ -3- fatty acids. Due to the high (213°C) smoking point of rice bran oil it is mixed with other vegetable oils to improve its cooking quality. Fibres are important as roughage and decide the glyciemic index of rice. However, higher content of fibres, reduces palatability.

Half of the world's population is suffering from one or more vitamin and/or mineral deficiency. Vitamin A, iron and zinc seem to be most prominent limiting components among them. Hence, varieties rich in micronutrients and vitamins will be the only sustainable solution to the problem of hidden hunger. In general, vitamin-A is absent in rice grains and micronutrients like iron and zinc are present in lower concentrations. Antioxidants are another important class of nutri-compounds that protect the cells from harmful effects of free radicals and reactive oxygen species released during various metabolic pathways of cells. Pigmented rice is rich in such compounds. The main phenolic compounds in pigmented rice are anthocyanins which are the major active components for antioxidation. Moreover, this rice is also rich in Vitamin B and E. Beside all these features, it also needs special mention that hundreds of end products have their specific quality requirements, which needs recognition and breeding interventions for further improvement.

#### 2.3. Genetic studies on quality traits

Following the advent of standard methods to measure different quality traits, studies on exploring their genetic basis were undertaken. Most of the quality traits were perceived as quantitative, except for aroma and grain pigmentation which were perceived as present-absent type and were studied in form of discrete ratios. Segregation ratio of aroma varied among different studies. However, the studies could unanimously conclude that aroma gene is recessive in nature which was later tagged by Ahn et al. (1992) as the fgr gene on long arm of chromosome 8. Bradbury et al. (2005) cloned the gene and characterized it further. In aromatic genotypes, a deletion in exon 7 of the gene coding for betaine aldehyde dehydrogenase (BADH2) generates a premature stop codon which results into loss of function of the gene (Shi et al. 2008) leading to accumulation of 2- Acetyl-1- Pyrroline (2AP). This is the main volatile compound (out of nearly hundred reported volatiles) imparting aroma to rice grain. Despite of being the most elaborately studied trait among all other quality traits, the quest for aroma is still on. The search for new variants of aroma gene, other minor genes and elucidation of its pathway are going on. The major focus of aromatic rice breeding in India was development of basmati rice varieties in which role of ICAR-IARI, New Delhi; CCSHAU, Hissar; PAU, Ludhiana and GBPUAT, Pantnagar were very crucial. However comparatively lesser focus was given in case of non-basmati aromatic rice both for basic and applied research. In a compendium prepared by the Indian Agricultural Research Institute (IARI) and APEDA, varieties such as Kalanamak, Tilak Chandan and Jeerabati (Uttar Pradesh), Kalajeera (Orissa), Katrani (Bihar), Ambemohar (Maharashtra), Gobindbhog and Badshahbhog (West Bengal), Dubraj, Badshahbhog and Jawaphool (Chhattisgarh) and Kalajoha (Assam) have been identified which could be harnessed and developed for their export potential. Many traditional aromatic rice genotypes viz. Bindli, Dubraj, Durgabhog, Makarkanda,



Badshabhog, etc. were reported to surpass basmati in one or more characteristics like aroma, texture, elongation on cooking and taste etc. and they mostly possess small to medium grain. Kalanamak and Dubraj were reported to command a premium price in the market. Breeding efforts for developing high yielding modern varieties of aromatic non-basmati rice have been quite limited when compared with intensive efforts for basmati. Mostly pureline selection and mutation approaches have been followed in their improvement. However the semi-dwarf version of Kalanamak landrace developed by researchers at IARI, New Delhi needs special mention. Chakraborty et al. (2016) explored the possible existence of any locus other than *BADH2* controlling aroma among 84 landraces of *indica* rice using functional marker (8-bp deletion) for *badh2* gene. Nearly 80 percent of the landraces carried the well-known *BADH2* deletion. However, eleven aromatic genotypes including wild ancestors lacked that particular functional allele. This indicated the existence of an alternate gene or allele controlling aroma in rice. Singh et al. (2010) however, have also established the role of *BADH1* gene and its haplotypes in controlling aroma in rice.

Like aroma, anthocyanin pigmentation in rice grain was also studied as a qualitative trait due to presence or absence type of phenotypes. Unlike aroma, this trait shows dominant gene control and two complementarily acting genes, Rc and Rd control the pericarp pigmentation. The Rd locus codes for dihydro flavonol reductase (DFR) enzyme and the Rc gene codes for Basic Helix-Loop-Helix (HLH) Protein. The Rc locus has been cloned and its three allelic variants have been well characterized. Its null allele (rc) with 14-bp deletion creates frame shift mutation and a premature stop codon leading to white pericarp phenotype (Brooks et al. 2008). Several pigmented rice genotypes have been identified and characterized in India, including the Chakhao rice of Manipur, Kalbahat rice of Maharashtra, Njavara of Kerala, etc. Njavara is well known for its medicinal significance in Ayurvedic medicines. However, the identified pigmented genotypes are low yielding and susceptible to lodging. Breeding interventions to improve their plant type and yield shall be highly beneficial in their popularization.

The quality traits other than aroma and grain pigmentation have been studied considering them as quantitative traits. Gene action studies on these quality traits lead to variable conclusions by different researchers. Advent of molecular markers has opened a new era of QTL and gene discoveries. More than 600 QTLs have been reported for different quality traits like chalkiness, cooking and eating quality, grain dimensions, pasting properties, grain protein content, iron and zinc (http://www.gramene.org). Table 2 summarizes the identified loci information of major quality traits and availability of their corresponding functional markers.

Detection of such large number of QTLs poses a great challenge for the plant breeder to efficiently and intelligently use this information for the genetic improvement of the traits. However, a comprehensive analysis suggests that the QTLs and genes involved in amylose biosynthesis pathway play a significant role in deciding the physical attributes (head rice recovery, chalkiness and grain dimensions) of grain along with its cooking and eating quality. *Waxy* (Wx) locus was first identified as the



Trait	Gene	FM Availability
Aroma/Fragrance	Badh2	Available
Amylose content (AC)	Wx	Available
Grain size	GS3	Available
Gelatinizationtemperature	SSIIa	Available
Iron (Fe)	OsYSL1, OsMTP1,OsFER1,OsFER2	Yet to be developed
Zinc (Zn)	OsARD2, OsIRT1OsNAS1, OsNAS2	
Fe and Zn	OsNASgene family	
	OsNAS3, OsNRAMP1,Heavy metal	
	iontransport, APRT	

Table 2: Genes and funct	tional markers for	quality traits in rice.
--------------------------	--------------------	-------------------------

Source: Adapted from Lau et al. (2015)

major locus controlling majority of the variation in amylose content in grain which in turn was known as the sole major factor affecting the cooking and eating quality of rice. The Wx locus codes for an enzyme Granule Bound Starch Synthase I (GBSS I) and has a minor effect in controlling gel consistency, but has no effect on gelatinization temperature. Alternate splicing of the Wx transcript leads to several allelic variations altering the degrees of amylose content in grain. The locus Alk is the major locus in controlling Gelatinization temperature and codes for the enzyme Soluble Starch Synthase IIa (Umemoto et al. 2005). Wx, SSIIa, SBE3 (Starch Branching Enzyme3), PUL (Pullulanase) have been reported to affect the different aspects of pasting properties in rice. Existence of cross talks and pleiotropy among the loci has also been reported. Cloning and characterization of several genes controlling grain appearance traits led to better understanding of their regulation and can therefore be utilized more efficiently in breeding programs. Chalkiness in the endosperm was attributed to the loose binding of the starch. However, among many QTLs reported for chalkiness only Chalk5 was isolated and characterized well (Li et al. 2014). Interestingly, some of the QTL clusters controlling grain dimensions (grain size and grain width) are reported to have pleiotropic effect with chalkiness.

Besides the above mentioned QTLs related to starch component of rice grains, a large number of QTLs have also been reported for the nutritional components like content of protein, iron, zinc etc. in grains. Expression of these traits is highly affected by growing environment and cultural practices followed to grow the crop. This makes their detection very difficult. Earlier, tight negative linkage was reported to exist between yield and grain protein content (GPC). In rice, many QTLs along with associated markers have been identified covering all 12 chromosomes for GPC among which chromosomes 1, 2 and 7 harbor most of the reported QTLs. The QTL *qPC1* present on long arm of chromosome 1 control GPC through its regulation of synthesis and accumulation of glutelins, prolamins, globulins, albumins and starch. It encodes a putative amino acid transporter (*OsAAP6*) and control GPC without affecting growth and grain yield suggesting that GPC and nutritional quality could be improved without reduction in grain yield (Peng et al. 2014). Several QTLs have been reported for higher



concentration of iron and zinc in rice grains, some of which have been listed in Table 2. However, all these studies need support of bioavailability data for the micronutrients. In the last decade, Indian researchers have paid major attention for improving the nutritional quality of rice grains especially protein, iron and zinc. Researchers from ICAR-IIRR, Hyderabad reported that wild relatives of rice such as *O. nivara*, *O. rufipogon*, *O.barthii*, and *O. longistminata*, and African cultivated rice *O. glaberrima* have higher level of Zn. The QTLs for Zn content were detected in Ch 3, 7 and 12 by using RIL population derived from Madhukar x Swarna. They also developed high grain zinc containing rice variety DRR Dhan 45 through conventional breeding. It is the first rice variety with high zinc content to be notified at national level with an average zinc content of 22.6 ppm in polished rice.

Significant progress has been achieved in development of transgenic lines especially in terms of nutritional quality in rice. However, their large scale application in form of cultivars is yet to be established. The biggest success in this regard was achieved in the development of beta-carotene rich golden rice which was developed by transformation of genes from daffodil and a soil bacterium Erwinia into rice and expressing them in the grain. The concentration of beta carotene was further improved by transforming the phytoene synthase gene from maize (Paine et al. 2005). Scientists from IARI, New Delhi have transferred the genes for golden rice traits in popular Indian rice varieties through backcross breeding strategy. In order to improve the iron content in grain, ferritin gene has been transferred from soybean into rice. Efforts have been made to reduce the phytic acid content which is a major anti-nutritional factor in rice grain. It declines the bioavailability of iron in body by chelating the same at low pH in the stomach. Simultaneously transgenics have been developed to improve the micronutrient content in grain to combat the problem of hidden hunger. Table 3 compiles the successful transformation events undertaken to improve the nutritional quality of rice grain. However, there is need to thoroughly analyze the stability of the events and their utility in trait transfer into high yielding genetic back grounds through breeding interventions.

S. No	Nutrient	Genes used
1	Vit A	Nppsy1, EucrtI,
	$\beta$ -carotene content	Daffodil Phytoene Synthase and Erwinia Phytoenedesaturase
2	Fe	<i>Osnas2</i> , , <i>Afphytase</i> , and <i>Osnas1</i> , <i>Osnas3</i> , <i>OsYSL2</i> , <i>Ferritin genes: SoyferH1</i> , <i>PyFerritin, rgMT</i> , <i>Gm ferritin</i> ,
3	Zn	Osnas2, Gm ferritin, AfphytaseandOsnas1
5	Fe and Zn	Nicotianamine synthase (NAS) genes [OsNAS1, OsNAS2, and OsNAS3, OsNAS3-D1, HvNAS1, AtNAS1 and HvNAS1], HvNAAT,HvNAAT-A, HvNAAT –B, Osfer2, SoyFerH1, SoyFerH2, Pvferritin, OsIRO2, OsYSL2, OsYSL15, HvNAS1, Afphytase,
23	Zn, Cu, and Ni	OsNAS3

Table 3. Genes used for transformation of rice to improve vitamin and mineral	
content.	

Source: Adapted from Mahender et al. (2016)

Due to increased incidences of diabetes in world population, development of rice genotypes with low glycemic index is receiving global attention. However the research in this particular area is still in nascent stage and remained limited to development of effective protocols and evaluation of limited set of germplasm. Any significant breakthrough in terms of detection of genes/QTLs for this trait is still not achieved. Although systematic breeding efforts for developing low glycemic index rice is in its nascent stage in India. Some varieties like Swarna (low GI reported by IRRI), Improved SambhaMahsuri (ICAR-IIRR, Hyderabad) and Madhuraj-55 (IGKV, Raipur) have been identified as low-GI-rice.

Besides all the traits discussed here, the 'Komal-Saul' (soft rice) of Assam with soak-and-eat property needs special mention. However, the unique property of this rice is highly location specific and expressed in just a few districts of north eastern India. Assam Agriculture University, Jorhat has released a few varieties of Komal-Dhan namely Aghonibora, Bhogalibora, etc. However, the underlying biochemical and molecular mechanism of this unique property is yet to be deciphered.

Indian rice breeding programmes are traditionally directed towards yield improvement. Targeted improvement for grain quality was carried out only in few cases. With a few exceptions, only advanced generations of high yielding materials are evaluated in nationwide trials for grain quality under All India Coordinated Rice Improvement Project (AICRIP). Many of the reported QTLs for different quality traits are either population specific, or control a minor portion of variation, or their expression is highly dependent on environment. As a result such QTLs are difficult to use by breeders. Sometimes the QTL region is so large that it becomes difficult to transfer the region intact. Therefore studies shall be carried out to identify and customize the QTLs and more preferably, the gene combinations that can be effectively utilized under target specific breeding programs. These QTLs and gene combinations will largely vary depending on the target environment and market, and therefore needs validation through multi environmental trials.

#### 2.4. Rice quality research at NRRI

The Institute was established in the backdrop of 'The Great Bengal famine' of 1942 caused due to huge yield loss by *Helminthosporium*, as a mitigation strategy to avoid such disasters further. Since its inception, the institution is incurred with the responsibility to feed millions of hungry bellies of an infant nation. Therefore the main focus from the beginning was to enhance yield. *Indica-japonica* hybridization program of FAO as well as trials of other introductions provided a very strong initial support for generation of valuable variations and development of high yielding promising genotypes. Rice cytology and genetic analysis of important traits contributed significantly in our present understanding of Rice science. Concern for rice quality along with yield got attention of visionary researchers even in days of immense pressure to feed the nation.



Breeding for fine grain began in 1953. Crosses were made to improve the grain characters of otherwise high yielding, highly adapted early maturing, coarse and red kernelled variety Ptb10 using pedigree method of breeding. In 1961, cultures of Xray irradiated Ptb10 were received which segregated for kernel color and were subjected to further testing. Several mutation breeding programs have been undertaken to improve grain quality of otherwise popular varieties like TN1, Taichung65, and CR2001. Neelabati was identified as a high yielding fine grained genotype recommended for cultivation in coastal/saline areas. Investigations on cooking quality of genotypes using the KOH test were initiated during 1963-64 and genotypes with superior cooking qualities were identified. As soon as a quick and reliable method to estimate the cooking quality was adapted, inheritance study was undertaken by crossing of contrasting genotypes. The inheritance study revealed that there are 2-3 genes that control cooking quality in rice and the trait can be subjected to genetic improvement. The study also indicated the possible role of minor genes for small modifications in trait expression. However varietal development program specifically focusing on the cooking and eating quality have not been undertaken yet.

Head Rice Recovery (HRR) percentage after hulling was also investigated during 1960s which ranged from 23.8 to 74.5%. Nature and texture of endosperm along with shape and size of grain were reported to influence HRR. Presence of abdominal white was found not to affect HRR. Highest recovery percentage (79.2) was reported in *Changsan* variety from Manipur. HRR percentage of japonica rice was not significantly different from that of *indica* types. Thorough study on the effect of time of harvesting after flowering, moisture content during harvesting on head rice recovery after milling was undertaken and it was observed that some genotypes like GEB24, T141, T90, etc. were more resistant to breakage when harvested within 30 days after flowering. However, ill filled kernels are a problem associated with early harvesting. The study could recommend that field harvesting after 30-40 DAF at 20-23% grain moisture level and shade drying to 11-13% moisture, gives highest hulling recovery by reducing the development of sun cracks in the kernels during field drying.

Inheritance of red kernel colour was studied during 1960s in segregating generation of a cross between GEB24 and Ptb10. PtB10 being well recognized for its superior grain type. Association with other traits was studied and their linkages established. Sanghamitra et al. (2017) reported low amylose content in purple rice genotypes, Chakhao, Mamihunger and Manipuri Black. Lower yield along with the low grain amylose content in these genotypes needs improvement through breeding. Significantly higher antioxidation property in the grains of these genotypes has been recognized. Higher heritability of the trait suggested greater scope for genetic improvement of anthocyanin and antioxidant content in pigmented rice.

ICAR-NRRI took important initiatives for genetic improvement of short grained aromatic rice. The aromatic landrace collections of the institute were characterized based on phenotypic descriptors and molecular markers (Roy et al. 2014 & 2016).



Study of variation in nuclear and chloroplast DNA sequences also provided a greater insight into the population structure and origin of aromatic landraces (Roy et al. 2016). Integration of participatory plant breeding with marker assisted pureline selection helped in better genetic gain from selection in three landraces Kalajeera, Machhakanta, and Haladichudi (Roy et al. 2017). Several superior pureline varieties like NuaKalajeera, NuaDhusara and NuaChinikamini were developed and released (Patnaik et al. 2014). Marker assisted pedigree selection helped to develop high yielding aromatic genotype, CR Sugandh Dhan-907 by crossing Pusa 44 and Dubraj (Patnaik et al. 2015). Besides aromatic short grain varieties, long slender grained genotypes with aroma (Poornabhog and Geetanjali) have been developed through mutation of basmati genotypes and were released for cultivation. Geetanjali variety is popular among the farmers and has been used for establishment of rice value chain in Odisha.

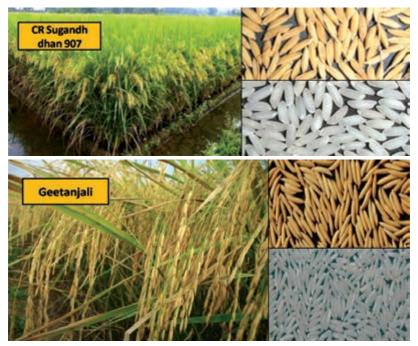


Fig.2. Field photograph of two aromatic rice varieties developed at ICAR-NRRI along with their respective grain type.

Protein in rice has ever been a major concern of rice researchers in past as well as in present due to its lowest grain protein content among cereals. Positive correlation of bran and aleurone layers with protein and thiamine content was identified and high protein genotypes were found to have a cellular patch in spermoderm (CRRI Annual Reports, 1949-50 and 1950-51). Studies on variation in protein content and aleurone layer thickness were undertaken in 450 representative genotypes of *japonica*, *indica* and *javanica* rice. Existence of positive correlation between aleurone layer thickness



and percent protein content in rice kernels was reported. Protein content in rice kernels varied from 6.1% to 10.1% while the aleurone layer thickness varied from 11.2µ to 75.0µ. Moreover, protein content in *japonica* rice was found to be higher than in white kernelled *indica* types. Inheritance of protein content was studied by the Rice Technology Section established during 1963 and they recorded frequency of occurrence of high protein genotypes among different duration classes of rice varieties in temperate and tropical zones. Due to 4-5% milling, a loss of 11-12% protein as compared to brown rice was recorded during 1966. Studies on inheritance of protein content suggested the role of polygenes in trait expression. Appearance of transgressive segregants in F<sub>2</sub> suggested the possibility to breed true for higher protein than in parents. Near infrared spectroscopy (NIR) was therefore calibrated and validated for large scale and high throughput phenotyping of GPC (Bagchi et al. 2015). Systematic breeding for high protein rice was initiated with identification of two landraces from Assam as donors. High protein trait was transferred to high yielding backgrounds of Naveen and Swarna through backcross breeding to release the first high protein variety in Naveen background as CR Dhan 310 (released by CVRC for Odisha, Uttar Pradesh and Madhya Pradesh) with 10.3% GPC in 2016. Another variety CR Dhan 311 has been released by State variety Release Committee of Odisha in the name of 'Mukul' with GPC of 10.1% and Zn content of 20 ppm. By using a backcross derived mapping population, a consistent OTL (qGPC1.1) over the seasons have also been identified.



Fig.3. Field photograph of two high protein rice genotypes developed at ICAR-NRRI

### 3. KNOWLEDGE GAPS

Amylose is known to be the major biochemical factor influencing majority of endosperm traits in rice. However sufficient variation for physico-chemical properties has been observed among genotypes having same amylose content. Later, amylopectin and protein content were also designated to play role in deciding the physico-chemical characteristics of grain. However, the factors taken together, sometimes fail to predict the physico-chemical quality of grain. Therefore there is need to discover some new factors that play significant role in deciding different aspects of grain quality along with those identified earlier.



Knowledge regarding biochemical and physiological mechanisms of superior grain quality needs to be generated for all quality traits. In case of aroma, though the gene and biochemical factors are well characterized. The biochemical pathway producing aroma is not well understood. Similarly, knowledge regarding the mechanism of higher accumulation of grain nitrogen in high protein rice is still to be deciphered.

It is well known that quality traits are highly affected by environment but very few studies have been undertaken on the effect of environmental factors on different quality traits. Moreover, the quantification of the environmental component on different quality traits is required to formulate mathematical equations to predict the trait expression in any environment more accurately.

Regarding the grain protein content, the mechanism of higher accumulation of grain protein along with the genes/QTLs having greater effect on the trait need to be identified. Molecular and physiological basis of micronutrient accumulation in grain requires further understanding. Stable sources of low phytic acid and high antioxidant content in rice need to be identified so that the problem of mal-nutrition can be overcome in more economic way. Researchers also need to give more emphasis to the non-conventional quality traits especially low Glycemic Index, Soak and Eat property, product making quality etc. as demand for the varieties carrying such properties are expected to increase further in coming years.

### 4. RESEARCH AND DEVELOPMENT NEEDS

- There is need to develop methods to precisely quantify the quality traits in a small sample volume without destroying it.
- There is need for intervention of engineers to design instruments for precise estimation of quality traits with more automation and thereby reducing variable human errors.
- More information on surrogate traits for different aspects of quality like grain type, cooking and eating quality, nutritional value etc. needs to be generated.
- Information on differential expression of quality traits under variable environments need to be generated to identify the environments for best expression of different quality traits. The information will support the breeders to evaluate and select genotypes for their breeding program in these environments.
- More QTLs related to quality working across genotypes and environments should be identified, characterized and cloned. Functional markers for such QTLs shall be highly beneficial for the breeders to undertake marker assisted breeding of quality traits.
- There is need to develop quality preference map for the country depending on quality preferences under different rice ecologies of different regions. This will support breeders to decide upon the quality traits to target in their respective



breeding programs. A global database needs to be developed that integrates the phenotyping data, genetic information and marker based genotyping data against all genotypes being studied. Such database shall be easy to handle so that the breeders can select the parents based on the available information.

### 5. WAY FORWARD

Since quality traits are highly influenced by environment (season/location/year), multi environment testing shall be an integral part of any quality breeding program. Quality evaluation data should always be supported with the GPS, agro-meteorological and soil quality data to improve the precision in quality breeding and provide a readymade information to the breeders to decide upon 'when and where' to select. Moreover, integrating the participatory approach of selection during the later stages of breeding program will facilitate in accelerated popularization of the developed varieties after release.

Popular landraces known for different quality traits shall be improved through appropriate breeding strategies to remove their critical limiting features for which farmers are compelled to stop their cultivation. Wild species may also be evaluated for their quality traits which may serve as useful donors for specific quality features. An elite population comprising of large number of fixed and intermating breeding lines with different permutations and combinations of quality QTLs/genes shall be developed in elite backgrounds. This will serve the need of elite genotypes for breeding in variable ecologies and provide a wide genetic base to avoid bottlenecks.

Discovery of new functional markers for QTLs and genes controlling quality in rice are very important so that the costly and destructive sampling methods for quality analysis may be avoided. However, exhaustive genotyping using the available marker information along with multi-location phenotyping must be undertaken among the genotypes selected for gene discovery. This will prevent unnecessary duplication of research (jumping into same QTLs/genes) and provide strong support for identification of new genetic factors. Moreover, these studies shall not remain limited to demarcating the QTL region, but should also characterize, clone and validate the genomic regions for their effective utilization in breeding programs. A global database for the genotypic and phenotypic data will be useful for selection of parents for breeding. Near isogenic lines (NILs) may be developed for these regions to study the effects of predicted genes individually and in combinations. Those single gene NILs and pyramided NILs will be very helpful to refine our understanding about the biochemical basis of quality. Rice quality breeding therefore, has a long way to go.

#### References

- Ahn SN, Bollich CN and Tanksley SD (1992) RFLP tagging of a gene for aroma in rice. Theoretical and Applied Genetics 84 (7-8): 825-828.
- Bagchi TB, Sharma S and Chattopadhyay K (2016) Development of NIRS models to predict protein and amylose content of brown rice and proximate compositions of rice bran. Food chemistry 191 (1): 21-27.



- Bradbury LM, Fitzgerald TL, Henry RJ, Jin Q and Waters DL (2005) The gene for fragrance in rice. Plant Biotechnology Journal, 3(3): 363-70.
- Brooks SA, Yan W, Jackson AK and Deren CW (2008) A natural mutation in *rc* reverts white-ricepericarp to red and results in a new, dominant, wild-type allele: *Rc-g*. Theoretical and Applied Genetics 117(4): 575-580.
- Chakraborty D, Deb D and Ray A (2016). An analysis of variation of the aroma gene in rice (Oryza sativa) Genetic resources and crop evolution 63(6): 953-959.
- Lau WC, Rafii MY, Ismail MR, Puteh A, Latif MA and Ramli A (2015) Review of functional markers for improving cooking, eating, and the nutritional qualities of rice. Frontiers in plant science 6: 832.
- Li Y, Fan C, Xing Y, Yun P, Luo L, Yan B, Peng B, Xie W, Wang G, Li X and Xiao J (2014) Chalk5 encodes a vacuolar H+-translocatingpyrophosphatase influencing grain chalkiness in rice. Nature genetics 46(4): 398-404.
- Mahender A, Anandan A, Pradhan SK and Pandit E (2016). Rice grain nutritional traits and their enhancement using relevant genes and QTLs through advanced approaches. Springerplus 5(1): 2086.
- Paine JA, Shipton CA, Chaggar S, Howells RM, Kennedy MJ, Vernon G, Wright SY, Hinchliffe E, Adams JL, Silverstone AL and Drake R (2005) Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nature biotechnology 23(4): 482-487.
- Patnaik A, Roy PS, Rao GJ, Panda D, Das SR, Patnaik SS, Jena M, Das KM, Singh ON 2014 NuaKalajeera, NuaDhusara and NuaChinikamini. Indian Journal of Genetics and Plant Breeding74 (4): 537-538.
- Patnaik A, Roy PS, Rao GJN, Patnaik SS and Sharma SG (2015) Genetic enhancement of Dubraj, a specialty rice through pedigree approach employing marker based selection for plant stature, aroma and grain quality. Euphytica 205 (3): 659-669.
- Peng B, Kong H, Li Y, Wang L, Zhong M, Sun L, Gao G, Zhang Q, Luo L, Wang G and Xie W (2014) OsAAP6 functions as an important regulator of grain protein content and nutritional quality in rice. Nature communications 11: 5.
- Roy PS, Jena S, Maharana A, Rao GJ and Patnaik SS (2014) Molecular characterization of short grain aromatic rice landraces of Odisha for detection of aroma. ORYZA-An International Journal on Rice 51(2):116-120.
- Roy PS, Patnaik A, Rao GJ, Patnaik SS, Chaudhury SS and Sharma SG (2017) Participatory and molecular marker assisted pure line selection for refinement of three premium rice landraces of Koraput, India. Agroecology and Sustainable Food Systems 41(2):167-185.
- Roy PS, Rao GJ, Jena S, Samal R, Patnaik A, Patnaik SS, Jambhulkar NN, Sharma S and Mohapatra T (2016) Nuclear and Chloroplast DNA Variation Provides Insights into Population Structure and Multiple Origin of Native Aromatic Rices of Odisha, India. Plos One 11(9): e0162268.
- Roy PS, Samal R, Rao GJ, Patnaik SS, Jambhulkar NN, Patnaik A and Mohapatra T (2016). Differentiation and description of aromatic short grain rice landraces of eastern Indian state of Odisha based on qualitative phenotypic descriptors. BMC ecology16: 36.
- Sanghamitra P, Bagchi TB, Sah RP, Sharma SG, Sarkar S and Basak N 2017 Characterization of red and purple-pericarp rice (Oryza sativa L.) based on physico-chemical and antioxidative properties of grains. Oryza (1) 54: 57-64.
- Shi W, Yang Y, Chen S and Xu M (2008) Discovery of a new fragrance allele and the development of functional markers for the breeding of fragrant rice varieties. Molecular Breeding 22 (2):185-192.



- Singh A, Singh PK, Singh R, Pandit A, Mahato AK, Gupta DK, Tyagi K, Singh AK, Singh NK and Sharma TR (2010) SNP haplotypes of the *BADH1* gene and their association with aroma in rice (*Oryza sativa* L.). Molecular Breeding 26 (2): 325-338.
- Umemoto T and Aoki N (2005) Single-nucleotide polymorphisms in rice starch synthase IIa that alter starch gelatinisation and starch association of the enzyme. Functional Plant Biology 32(9): 763-768.
- Yang Y, Guo M, Li R, Shen L, Wang W, Liu M, Zhu Q, Hu Z, He Q, Xue Y and Tang S (2015) Identification of quantitative trait loci responsible for rice grain protein content using chromosome segment substitution lines and fine mapping of *qPC-1* in rice (Oryza sativa L.). Molecular Breeding 35(6): 130.<sup></sup>



# Genetic Improvement of Rainfed Shallowlowland Rice for Higher Yield and Climate Resilience

#### SK Pradhan, M Chakraborti, K Chakraborty, L Behera, J Meher, HN Subudhi, SK Mishra, E Pandit and JN Reddy

#### **SUMMARY**

In India, 16 million ha area is under rainfed lowland rice cultivation. The rainfed shallow lowland is characterized by water accumulation of 0-50 cm and faces frequent drought and flash floods. Rice crop faces several challenges when grown under shallow lowland ecology. Simultaneous incorporation of drought and submergence tolerance besides imparting lodging resistance, anaerobic germination ability, seed dormancy and tolerance to major biotic and abiotic stresses becomes very crucial for developing rice varieties for shallow lowlands. Well-characterized genes and QTLs are available for majority of traits to be deployed in suitable combinations for resilient breeding. Updated information on genetic improvement of shallow lowland rice for attaining higher productivity has been discussed in this chapter.

#### **1. INTRODUCTION**

In India, rice is grown in 43 Mha, and approximately 50% of these areas are under rainfed ecology with low productivity due to various abiotic and biotic stresses. The severity of biotic and abiotic stresses is changing frequently due to the effects of climate change. The rainfed lowland ecologies cover around 16 Mha of which 92% are located in the eastern region of the country. Depending upon the water depth and duration of water logging in the lowland ecology during growth period of rice, it has been classified into rainfed shallow lowland, semi-deep, deep and very deep water or floating type ecosystem. The rainfed shallow lowland is characterized by water accumulation of 0-50 cm that face frequent drought and flash flood. As these areas are not suitable for growing most of the other economically important crops, developing high yielding climate resilient varieties of rice needs to be increased from lesser land area. In fact with the increasing scarcity of water for rice cultivation, the future thrust for growing rice must focus with such ecologies.

Rice crop faces several challenges when grown under shallow lowland ecology. Many rice growers under rainfed shallow lowlands adopt direct seeding of rice. These direct seeded fields usually not properly leveled before seed sowing. After sowing the plots get severe rain water accumulation frequently and resulting in heavy seedling mortality and hence low yield. Presence of stagnant water for more than 10 days period is very common in most areas under this ecology. Although by its name, the



ecology seems to possess sufficient amount of water and stagnant water seems to create problems, occasional drought spells are also observed. As the crop is grown almost completely under rainfed conditions, scope for supplementary irrigation is very much limited. Besides, the genotypes suitable for shallow lowland ecologies have longer maturity duration (140 days or more) and comparatively higher plant heights. In the eastern part of the country where these varieties are grown, frequent incidences of untimely rainfall and storms during grain maturity lead to heavy crop loss caused by lodging and pre-harvest germination of seeds. Biotic stresses like stem borer, brown plant hopper, gall midge, leaf folder, bacterial blight, bacterial sheath blight and false smut are significantly affecting rice grain yield. Simulation studies have clearly shown that, in future the distribution pattern of rains or associated wind is expected to show more erratic behavior (Turner and Annamalai, 2012). Moreover, disposal of paddy straw biomass is now-a-days become a burning issue and environmental concern. Keeping the context of the country in terms of utilization of straw, it will also be necessary to develop lodging resistant cultivars without compromising feed quality and biodegradability. Thus, there is an urgent need of region specific climate smart breeding in mega rice varieties with stacking of various stress tolerance genes through multipronged approaches. Hence, simultaneous incorporation of drought and submergence tolerance besides imparting lodging resistance, anaerobic germination ability, seed dormancy and tolerance to major biotic and abiotic stresses becomes very crucial for developing future rice varieties for shallow lowlands.

Fortunately rice researchers have already discovered many such useful genes and QTLs. A major QTL *Sub1* is very useful for conferring submergence tolerance for 12 days. Many yield QTLs have been reported which works well under drought stress. Pyramiding of these QTLs is expected to increase grain yield in rice under drought stress. Though no host plant resistance genes are identified for stem borer, but more than 30 resistance genes/QTLs are reported for brown plant hopper host plant resistance. Till date, 42 resistant genes have been reported for controlling bacterial blight disease in rice. Many yield enhancing QTLs have already been cloned in rice. Two genes controlling anaerobic germination are already known. Now it will be interesting to see how these QTLs or genes will behave when they are all taken together in a single background.

The impact of the Green Revolution has sidelined the eastern Indian rainfed shallow lowlands. Sizeable rice areas of eastern India are under shallow lowlands with low production. We have to feed the burgeoning population in near future. The irrigated ecology has shown symptoms of yield plateauing. Thus, this traditionally neglected ecosystem has got tremendous potential to increase the yield level and total production as a whole.

Keeping these factors in mind, this chapter deals with the up-to-date information on genetic improvement of shallow lowlands rice for attaining higher productivity. Prominent genetic approaches for obtaining higher productivity against various abiotic and biotic stresses in this ecology namely submergence, drought, anaerobic



germination, brown plant hopper, gall midge, leaf folder, bacterial leaf blight, bacterial sheath blight, false smut, lodging resistance, dormancy, yield etc. are discussed here.

### 2. STATUS OF RESEARCH

Submergence and drought are two major abiotic stresses in rainfed rice cultivation. Rice plants tolerant to complete submergence usually exhibit very limited elongation during submergence and often show tolerance to complete flooding, a strategy known as quiescence. The mechanistic understanding of molecular regulation of true submergence tolerance/ quiescence in rice has been advanced through functional characterization of key genes responsible for acclimatization to submergence stress in rice (Xu et al. 2006). Limited number of rice genotypes possess inherent mechanism to tolerate a deep transient flash flood through economization of energy reserves (quiescence strategy) (Fukao and Xiong 2013). Quantitative trait locus (QTL) analysis and map-based cloning revealed that the SUBMERGENCE1 (SUB1) locus, encoding a variable cluster of two or three tandem-repeated group VII of ETHYLENE RESPONSIVE FACTOR (ERF-VII), regulate the quiescence response (Xu et al. 2006). Most of the reported rice accessions found to contain SUB1B and SUB1C genes at the SUB1 locus, whereas SUB1A was reported to contribute ~70% of submergence tolerance to some *indica* and *aus* rice varieties. The major QTL, Sub1A has been fine mapped on chromosome 9 in the submergence tolerant cultivar FR13A (Xu et al. 2000). Researchers at International Rice Research Institute (IRRI), Philippines used back crossing involving a double haploid derived from three tolerant parents (FR13A, IR49830-7-1-2-2 and IR67819F2-AC-61) and a japonica rice KDML105. They were able to develop new Jasmine rice carrying QTL for submergence tolerance retaining the quality traits of KDML105. Under considerable stagnation of water, no other cereals besides rice can survive and produce. This unique ability in rice is attributed to its ability to elongate rapidly with onset of water stagnation. Under both the situations ethylene responsive factor genes control the elongation but in opposite direction i.e. quiescence and elongation (expansion) (Hattori et al. 2009). Snorkel 1 (SK1) and Snorkel 2 (SK2) allow rice to elongate fast whereas Submergence1A-1 (Sub1A-1) allows rice to squeeze elongation for adaptation to water stagnation and flash floods conditions, respectively. Both SK genes and Sub1A encode ethyleneresponsive factor, a specific group of transcription factor related to gibberellin biosynthesis or signal transduction. Several mega rice varieties, which were submergence sensitive were being converted to submergence tolerant types through introgression of Sub1A-1 genes through marker-assisted backcrossing and released for commercial cultivation in different submergence prone areas of Asia and Africa.

Drought is a major yield limiting factor in rainfed lowlands. Progress in drought breeding is very slow. The recent scenario in climate change indicates more unpredictable drought intensity in the eastern region of the country directing us for developing drought-resilient varieties. Fukai and Cooper (2001) have summarized the complexity of drought tolerance and emphasized strategies that influence yield under drought stress. The use of grain yield under drought stress is a selection criterion



which is useful for developing high-yielding rice cultivars for rainfed rice-growing areas. Several major quantitative trait loci (QTLs) showing effects under drought need to be pyramided together to develop drought-tolerant versions of popular drought-susceptible varieties. Three QTLs namely qDTY1.1, qDTY2.1 and qDTY3.1 are showing yield improvement under drought stress (Dixit et al. 2014). The QTL, qDTY1.1, shows a consistent effect on grain yield under lowland drought. Two large-effect QTLs influencing grain yield (qDTY2.1 and qDTY3.1) show  $R^2$  values of 16.3% and 30.7%, respectively, under lowland drought. Another QTL, qDTY12.1 shows consistent additive effects of 45% under drought, is mapped within the physical interval of 2.7MB between two microsatellite markers RM28048-RM28166 (Dixit et al. 2014). A large effect QTL (qDTY3.2) shows positive effect on yield under drought stress which co-localizes with Hd9, a locus which is related to days to flowering.

*Dro1* is negatively regulated by auxin and is involved in cell elongation in the root tip that causes asymmetric root growth and downward bending of the root in response to gravity. Higher expression of *Dro1* increases the root growth angle, whereby roots grow in a more downward direction. Introducing *Dro1* into a shallow-rooting rice cultivar by backcrossing enabled the resulting line to avoid drought by increasing deep rooting, which maintained high yield performance under drought conditions relative to the recipient cultivar. Root growth angle (RGA) is an important trait that influences the ability of rice to avoid drought stress. *DEEPER ROOTING 1 (Dro1)*, which is a major quantitative trait locus (QTL) for RGA, is responsible for the difference in RGA between the shallow-rooting cultivar IR64 and the deep-rooting cultivar Kinandang Patong (Uga et al. 2013). Natural variation in RGA in rice cultivars carrying functional *Dro1* alleles may be controlled by a few major QTLs and by several additional minor QTLs (Kitomi et al. 2015).

Rice is usually grown as direct seeded crop in the rainfed shallow lowlands of eastern India, which frequently coincides with heavy rainfall in a poorly leveled and with poorly drainage field resulting in poor plant stand. Submergence just after sowing imposes stress by creating hypoxic condition (3% Oxygen) during germination as well as during vegetative stage. Interestingly, mode of overcoming hypoxic stress by rice plants seems to be different during germination and vegetative stages. The genes and QTLs reported for vegetative stage submergence tolerance are of no use to tolerate germination stage submergence and vice-versa. Being adapted to aquatic ecology, rice has developed the unique mechanism to germinate and extend its coleoptile under water even in complete absence of oxygen - a phenomenon termed as anaerobic germination (AG). In general, rice coleoptile under water has been found to elongate about 1 mm h<sup>-1</sup> to reach the atmosphere by rapid elongation of basal cells (up to 200 im in 12 h) immediately after emerging from embryo (Narsai et al. 2015). However, anaerobic germination potential (AGP) varies greatly among different rice genotypes, which ultimately provide an edge to a few genotypes to perform better under oxygen deficient conditions over others.

Anaerobic respiration usually yields much less energy as compared to the aerobic mode of respiration. Here, the energy requirement is largely fulfilled by glycolysis followed by alcoholic fermentation. Transcriptome analysis data also revealed up-

regulation of genes related to starch and glucose metabolism, glycolysis and fermentation during germination under anaerobic condition/submergence (Narsai et al. 2017). Starch degrading enzymes like  $\alpha$ -amylase, aldolaseand sucrose synthase are up regulated in germination stage oxygen deficiency (GSOD) tolerant cultivars greatly compared to susceptible cultivars with higher RAmy 3D gene expression as well as greater up-regulation of rice cytosolic hexokinase *OsHXK7* (Kim et al., 2016). The work of mapping QTLs imparting high anaerobic germination potential (AGP) has been initiated and one of the identified QTL, *qAG-9.2* has been fine-mapped to *OsTPP7* gene which encodes trehalose-6-phosphate phosphatase involved in starch mobilization during germination (Kretzschmar et al. 2015). Recent studies showed effective operation of anaerobic respiration and nitrogen metabolism in tolerant rice genotypes led to more energy efficient metabolic system under oxygen limiting GSOD condition resulting in better ROS handling and cellular pH maintenance (Vijayan et al. 2018).

Phosphorus is a limiting nutrient in the direct seeded rice ecology. Due to high cost of phosphatic fertilizers, farmers are not applying required quantity of the fertilizer. A major QTL *Pup1*, located on chromosome 12 exhibiting 78.8 % of the total phenotypic variance for phosphorus uptake has been found to be associated with tolerance to P deficiency and efficient P uptake in low phosphorus soil (Wissuwa et al. 2002). Kasalath, a *Pup1* donor variety has a 278 Kb INDEL and near isogenic lines with the QTL exhibited an increase P uptake and also 2-to 4- fold increase in grain weight per plant (Chin et al. 2010). Therefore, the development of P-efficient crop varieties that can grow and yield better with low P supply is a key to improve rice production.

Studies have showed that after flowering, lodging one day earlier causes yield loss to the tune of 2.6-2.7% per day in best *japonica* varieties grown at China. Through systematic studies on lodging resistance from last 25 years; researchers from Japan were able to demonstrate that lodging resistance can be significantly improved in rice without any compromise on grain yield. In fact they were able to identify mechanisms which simultaneously improve yield and lodging resistance and the genes were further cloned and characterized (Yano et al. 2015). Typhoon causes major damage to rice crop in Japan especially at maturity stage. Researchers were able to develop QTL-NILs by transfer of a genomic region from an Indian landrace Kasalath in the background of *japonica* cultivar Koshihikari which could survive two or more moderate typhoons in a particular year without any reduction in yield (Ishimaru 2008). The same research group was also able to identify another functional QTL which could improve lodging resistance through prevention of factors which lead to culm strength deterioration after grain lling (Kashiwagi et al. 2016). The prospect of combining high biomass production and lodging resistance was demonstrated through development of a long-culm rice forage cultivar named as 'Leaf Star' which was a low-lignin producing lodging resistant rice cultivar suitable for feed and bioenergy production (Ookawa et al. 2014). The important QTLs/genes responsible for lodging resistance in rice are presented in Table 1. The possibility of effective utilization of lodging resistance in rice while addressing the concern of yield and feed quality in Indian rice breeding programme need to be considered.



QTL/ Gene	Feature	Population	Reference				
<i>bsuc11</i> (Breaking strength upper culm 11)	Present in Chromosome 11. Improves Breaking strength of upper culm through thickening of cortical bre tissues in internode and raising the level of holocellulose.	Chromosome Segment Substitution Lines (CSSLs) developed from 'Koshihikari' and 'Kasalath'	Kashiwagi 2014				
<i>prl5</i> (Pushing resistance lower 5)	Present in chromosome 5. Improves pushing resistance of lower plant parts ( <i>prl5</i> ) by delaying senescence and increasing carbohydrate reaccumulation in stems.	CSSLs developed from 'Koshihikari' and 'Kasalath'	Kashiwagi and Ishimaru 2004				
<i>lrt5</i> (Lodging resistance in atyphoon 5)	Plants carrying this QTL circumvent domino effect. Starch content of upper culm in <i>lrt5-NILs</i> was 4.8 times when compared with recurrent parents	CSSLs developed from 'Koshihikari' and 'Kasalath'	Ishimaru et al. 2008				
SCM2 (Strong Culm 2) QTL/ Aberrant Panicle Organization1 (APOI) gene	The gene simultaneously improves section modulus of culm along with grain number per panicle.	CSSLs derived from Sasanishiki and Habataki	Ookawa et al. 2010				
SCM3 (Strong Culm 3) QTL/ Teosinte Branched1 (OsTB1) gene	The gene simultaneously improves section modulus of culm along with grain number per panicle. NILs of <i>SCM2</i> + <i>SCM3</i> further improves both traits	Backcross Inbred Lines (BILs) of Koshihikari and Chugoku117	Yano et al. 2015				

Under shallow lowland condition, seed dormancy is a prerequisite for breeding of cultivars as it prevents pre-harvest sprouting of seeds which in terms significantly reduce the quality and storability of the harvest. Pre-harvest sprouting mostly happens when humidity and temperatures increase during grain filling and maturation, which are caused mostly by unseasonal rains. Several efforts for mapping QTLs/genes for seed dormancy have been taken by researchers of China and Japan using *indica* x *japonica* derivatives. Some of the significant findings have been listed in Table 2.

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the most important disease of lowland rice in India. In some areas of Asia, it can reduce crop yield by up to 50% (Khush et al. 1989) or even up to 80% (Singh et al. 1977). In absence of effective chemical or other control agents against the pathogen, host plant resistance has gained enormous importance in controlling this disease. Using the gene pyramid approach, improved *indica* rice cultivars with broad spectrum durable BB resistance have been developed by combining different genes (Pradhan



#### Table 2. List of QTLs/ genes identified to confer seed dormancy in rice.

Ge	enes/QTLs detected	Population	Reference
	One QTL ( <i>Sdr1</i> ) for seed dormancy was identified which was very tightly linked to another QTL for heading date ( <i>Hd8</i> )	BILs from Nipponbare and Kasalath	Takeuchi et al. 2003
2.	Four major QTLs and several minor QTLs were detected. qSD-3 in chromosome 3 was most important.	Doubled haploid from indica x japonica	Guo et al. 2004
3.	Three QTLs ( <i>Sdr6</i> , <i>Sdr9</i> and <i>Sdr10</i> ) were detected	CSSLs of Nona Bokra and Koshihikari	Marzougui et al. 2012
4.	Four putative QTLs detected. <i>qSD-6.1</i> most effective among those.	BIL of Nipponbare x Kasalath	Sasaki et al. 2013
5.	Eight additive effect QTLs for seed dormancy detected. Best combination for pyramiding also suggested.	RILs of japonica x indica	Wang et al. 2014
6.	Several QTLs were mapped at 4, 5 and 6 weeks after heading in the same population	RILs of japonica x indica	Cheng et al. 2014
7.	Sixteen and 38 loci significantly associated with dormancy in freshly harvested seeds and after ripened seeds were detected. There were three common QTLs among them.	Association mapping in global accessions of rice	Magwa et al. 2016
8.	Six major additive effect QTLs ( <i>qSD3-2</i> , <i>qSD4-1</i> , <i>qSD7-1</i> , <i>qSD7-2</i> , <i>qSD7-3</i> and <i>qSD11-2</i> ) with contributions of more than or equal to 30 percent. Validated over seven cropping seasons	Single Segment Substitution Line of one indica rice with several donors	Zhou et al. 2017
9.	Ten SNPs were identified which significantly affect Pre Harvest Sprouting and the alleles were validated using regression based model.	Resequencing of multiple accessions.	Lee et al. 2017

et al. 2015; 2016). A three-gene combination appeared to be the most effective; with *Xa21* contributing the largest component of resistance. Gall midge insect is an internal feeder of rice plant and it reduces rice yield severely. Till date, 11 gall midge resistant genes have been identified.

As overall grain yield of rice from a crop field is a very complex trait for direct improvement; efforts have been taken worldwide for utilizing the genes and QTLs for its component traits. *Gn1a*, a major QTL, has been cloned and characterized for grain number per panicle. The functional analysis shows that *Gn1a* encoded cytokinin oxidase/dehydrogenase (Ashikari et al. 2005). Ideal plant architecture1 (*IPA1*) changes rice plant architecture and enhances rice grain yield. This IPA1 encodes OsSPL14 (Jiao et al. 2010). The genes/QTLs associated with the yield enhancing traits are presented in Table 3.



# Table 3. Useful genes/QTLs for pyramiding of yield component traits for increasing yield potential in rice.

Yield Genes/		Chromosome	Donor line/		
QTLs	Traits	location	variety	Gene function	Reference
1. Gn1a	Grain number	1	Habataki	Cytokinin Oxidase/ Dehydrogenase2	Ashikari et al. 2005
2. SPL16	Panicle branching	8	ST12	Squamosa Promoter binding protein like-14	Wang et al. 2012
3. SCM2	Culm diameter	6	Habataki	F-box containing protein	Ookawa et al. 2010
4. Ghd7	Delayed heading date and increase plant height and yield		Minghui63	CCT domain protein	Xue et al. 2008
5. GS5	Grain length	5	Zonhhua11	Serine carboxy peptidase	Li et al. 2011
6. GW5	Grain weight	5	Nipponbare	Unknown protein	Weng et al. 2008
7. TGW6	Test grain weigh	t 6	Kasalath	IAA glucose hydrolase	Ishimaru et al. 2013
8. DEP1	Erect panicle, high grain numbe	9 er	Shenung265	Phosphatidy- lethanol-amine binding protein	Huang et al. 2009
9. SPIKE1	Grain number/ panicle	4	IR68522-10 -2-2	Polar auxin protein	Fujita et al. 2013
10.GW2	Grain weight	2	Oochikara	Ring-type E3 ligase protein phosphatase	Song et al. 2007
11. GS3	Grain length	3	Minghui63	Trans membrane protein	Fan et al. 2006

FR13A, a selection from landrace 'Dhalaputia' is a widely used submergence tolerance donor line in submergence breeding programme. This line is the source material for submergence tolerance QTL, *Sub1*. The variety Swarna in which *Sub1* has been introduced through marker-assisted breeding has become very popular in rainfed lowland ecologies in the country. This was the first ever submergence tolerant variety released in the country (Neeraja et al., 2007). Swarna-Sub1 was released by SVRC, Odisha and SVRC, Uttar Pradesh and notified by Dept. Of Agriculture and Cooperation, Ministry of Agriculture, Govt. of India. Many pyramiding works on bacterial blight has also been performed in the country in various superior backgrounds (Pradhan et al. 2015; 2016). Till date 11 gall midge resistance genes have



been identified (Dutta et al. 2014). Researchers of ICAR-Indian Institute of Rice Research developed drought tolerant variety DRR Dhan 42 through pyramiding of *qDTY1.1, qDTY2.1* and *qDTY3.1* in IR64 background. The donor germplassm line N22, CR143-2-2, Bala, Lalnakanda, Dagad Deshi, Moroberekan, Aus 276, Vandana, Apo and IR55419-04 are commonly used donors for drought tolerance breeding programme in the country.

The rice breeders in India have mostly depended upon dwarfing genes for improving lodging resistance coupled with phenotypic selection for stronger culm. The general perception remained that selection of high culm strength may have negative impact on yield besides its low acceptability among farmers due to reduced feed quality of straw. There is no significant research report about genetic studies on lodging resistance of rice from India in last 20 years especially for waterlogged ecologies. Only very recently a report for mapping QTLs associated with lodging resistance in dry direct-seeded rice have been published by researchers from IRRI, India hub (Yadav et al. 2017). Rathi et al. (2011) mapped two QTLs for seed dormancy and one for duration of seed dormancy in a smaller  $F_2$ population derived from two *indica* genotypes.

The submergence tolerance property of FR13A was first reported by NRRI and shared with other institutes, which was subsequently mapped as Sub1 and used in rice breeding. The ICAR-NRRI has developed pyramided lines with Xa21, xa13 and xa5 resistance genes in the backgrounds of Swarna, Jalmagna, IR 64, Lalat and Tapaswini. The Institute also collaborated with IRRI for release of Swarna-Sub1 through SVRC, Odisha. Under 'QTL to variety' project, the Institute has stacked three abiotic stress tolerance genes viz., Sub1+ qDTY1.1+qDTY2.1 in the background of Swarna variety. Also, under IRRI-NRRI collaborative project, Swarna variety containing Sub1, qDTY1.1, qDTY2.1 and qDTY3.1 QTLs have been released for submergence and drought affected areas of Andhra Pradesh, Telangana, Odisha, Uttar Pradesh and West Bengal states of the country. A bacterial blight resistance genes pyramided line of Swarna has been released as CR Dhan 800 by SVRC, Odisha. Efforts are made to improve the popular lowland varieties like Gayatri, Sarala, Varshadha, Pooja and Pratikshya with submergence tolerance by incorporating "Sub1" gene through markerassisted backcross breeding. Presently, the improved lines of these varieties are in the advanced stages of testing. Closely linked markers have been identified for gall midge resistance gene Gm4 and QTL for BPH resistance (Mohanty et al. 2017) which can be used for pyramiding with other genes reported nationally and internationally. The Institute has released several varieties namely Pooja, Sarala, CR Dhan 500, CR Dhan 401, Gayatri, Savitri, Dharitri, Swarna-Sub1, CR Dhan 800, CR Dhan 801, CR Dhan 505, CR Dhan 506 and CR Dhan 508 for cultivation under shallow lowland ecosystem (Table 4).



				Potential			
Variet		Year of release	Maturity duration	yield (t ha <sup>-1</sup> )	Grain type*	Special feature**	Recommended States
	namika	1979	145	4.5	LB	-	Tamil Nadu
	amakrishna	1980	130	4.0	MS	BLB, GM, tolerant to water logging, iron toxicity	Odisha
3. Sa	amalei	1980	150	4.5	LS	GM, BL	Odisha
4. Sa	avitri	1982	155	5.5	SB	BL, BB, MR	Andhra Pradesh, Odisha,Tamil Nadhu
5. Dl	haritri	1988	150	5.0	SB	BI, BB, SB, GM	Odisha
6. Pa	admini	1988	145	4.0	ShS	BB	Odisha
7. M	loti	1988	145	5.0	LS	BL, RTV, GLH	Odisha
8. CI	R 1002	1992	145	4.5-5.0	SB	ShB,GLH	West Bengal, Odisha, Bihar
9. Se	eema	1992	150	4.5-5.0	MS	Blast, BPH, GM, tolerant to water logging	Odisha
10. Pc	ooja	2000	150	5.0	MS	BL	Odisha, Madhya Pradesh, West Bengal
11. Ke	etekijoha	2005	145	3.5	MS	BB, ShB, SB, GM	Odisha
12. Ni	ua Kalajeera	2008	145-150	3.0	SB	YSB, BI, Sh.R, Black husk	Odisha
13. Ni	ua Dhusara	2008	145-150	3.0	SM	NB, ShR, RTV, GM	Odisha
14. Sv	warna- Sub1	2009	143	7.0	MS	Tolerant to complete submergence for two weeks	Odisha, Uttar Pradesh
15. Re	eeta	2010	150	7.5	MS	BL, NB, BS, ShB, ShR, SB, LF	Odisha, West Bengal, Tamil Nadhu, Andhra Pradesh
16. Ni	ua Chinikamini	2010	145-150	3.5	SB	RTV, SB, RGM, NB, Sh.R	Odisha
17. CI	R Dhan 500	2011	160	7.2	MS	MR to LB, NB, GM, SB, LF	Odisha,Uttar Pradesh
18. Su	umit	2012	145-150	7.5	LB	LB,SB,LF	Odisha
19. Pc	oorna Bhog	2012	140-145	5.2	LS	NB, GM, ShR, SB	Odisha
20. Ja	lamani	2012	160	4.9	MS	MR-LF, GLH, LB, NB, BS, G, SB	Odisha

Contd....



			Potential			
Variety name	Year of release	Maturity duration	yield (t ha <sup>-1</sup> )	Grain type*	Special feature**	Recommended States
21. Jayantidhan	2012	160	5.3	MS	MR-LB, NB, ShB, ShR, RTV, GM, SB, LF, RT, WM	Odisha
22. CR Dhan 407	2014	150	5.8	LB	MR to leaf blast, neck blast	Odisha, West Bengal
23. CR Dhan 505	2014	162	7.1	MS	Submergence tolerance	Odisha, Assam
24. CR Dhan 408 (Chakaakhi)	2014	165	7.5	LB	MR-LB, NB, BB, Sh.R, SB, LF, WBPH	Odisha
25. CR Dhan 508	2016	160	8.5	LB	MR-NB, BS, ShR, SBR, LF, WM	Odisha, Assam West Bengal
26. Pradhandhan (CR Dhan 409)	2016	160	7.8	LS	MR-LB, NB, ShR, SBR, LF	Odisha
27. CR Dhan 507	2016	165	6.8	MB	MR-NB, BS, ShR, ShB, SBR, WM, LF	Odisha
28. CR Dhan 910	2016	140-145	5.6	MS	MR-BL,NBl, ShR, RTV, SB, LF, WBPH	Odisha
29. CR Dhan 909	2017	140	6.5	MS	Tolerant to LB, NB, Sh.R, RTD	Uttar Pradesh, Bihar, Assam, Maharashtra
30. CR Dhan 801	2017	140	7.8	MS	For submergence and drought prone areas	Uttar Pradesh, Bihar, Odisha, West Bengal

Note: \*SB-Short bold; LS-long slender; MS-Medium slender; MB-Medium bold; LB-Long bold\*\*Blblast; BB-Bacterial blight; BPH-brown plant hopper; BS-brown spot; GB-gundhi bug; GM-gall midge; GLH-green leaf hopper; LF-leaf folder; RTV-rice tungro virus; SB-stem borer; Sh.B-Sheath blight; Sh.R-Sheath rot; WBPH-white backed plant hopper; YSB-yellow stem borer; HS-Helmenthosporium

#### 3. WAY FORWARD

Research need to be carried out to reduce the yield limiting factors of the rainfed lowland ecology. Efforts to increase yield potential of the shallow lowland rice may further be expedited to get a quantum jump in yield from a vast neglected area of eastern India. As the rainfed areas of the country are highly affected by climate change effects, the high yielding varieties of the ecology need to be stacked with stress tolerance gene(s)/QTLs for making them climate smart. Effects of the QTLs/ gene(s) need to be segregated out first and then combination effects need to be studied so that proper pyramiding as well as stacking combinations can be identified. Study on development of host plant resistance for the emerging diseases and insect pests need to be taken up in an environment friendly manner. Translocation of elements like heavy metals from soil to grains and straw need to be in a safer level. Genetic enhancement of the materials for toxic and other problematic soils need more attention



in future. Trait combinations in superior background should lead to development of high and sustainable performing varieties with higher benefits to the producers. Thus, in popular varieties, multiple tolerance genes for submergence, anerobic germination, yield QTLs under drought, bacterial blight resistance genes, gall midge resistance genes, seed dormancy and yield enhancing QTLs need to be stacked to make them highly resilient to the climate change.

In future, production of rice needs to be increased from lesser land area. For this purpose there is effort towards increasing per plant yield though higher grain number per panicle and grain weight. This will definitely increase the weight of upper part of the rice plant and thereby increasing the chance of lodging with current plant types of *indica* rice. The problem is further coupled by increasing climatic vagaries like erratic rainfall, increase in extreme weather events and uncharacteristic wind flow especially in eastern and southern coasts of the country. Lodging related losses in semi dwarf varieties still remains to the extent of 35% and at some specific locations even >85% where traditional cultivars are grown. There is a need for systematic studies for improving lodging resistance of rice in our country. More so, understanding the mechanism and devising strategies for improvement of lodging resistance to *indica* rice would be important to breed future rice with high biomass coupled with higher harvest index. Further it will be necessary to save rice in climate vulnerable regions where incidences of sudden heavy rain or wind during maturity of crop cause severe production losses.

#### References

- Ashikari M, Sakakibara H, Lin S, Yamamoto T, Takashi T, Nishimura A, Angeles ER, Qian Q, Kitano H and Matsuoka M (2005) Cytokinin oxidase regulates rice grain production. Science 309:741-745.
- Cheng J, Wang L, Du W, Lai Y, Huang X, Wang Z and Zhang H (2014) Dynamic quantitative trait locus analysis of seed dormancy at three development stages in rice. Molecular Breding 34(2):501-10.
- Chin JH, Lu X, Haefele SM, Gamuyao R, Ismail A, Wissuwa M and Heuer S (2010) Development and application of gene-based markers for the major rice QTL Phosphorus uptake 1. Theoritical and Applied Genetics 120:1073–1086.
- Dixit S, Singh A and Kumar A (2014) Rice Breeding for High Grain Yield under Drought: A Strategic Solution to a Complex Problem. International Journal of Agronomy http://dx.doi.org/ 10.1155/2014/863683.
- Dutta SS, Divya D, Durga RV, Reddy DT, Visalakshmi V, Cheralu C, Singh IK and Bentur JS (2014) Characterization of gall midge resistant rice genotypes using resistance gene specific markers. Journal of Experimental Biology and Agricultural Sciences 2(4):439-445.
- Fan N, Xu R, Huang L, Zhang B, Duan P, Li N, Luo Y and Li Y (2016) Small grain11 controls grain size, grain number and grain yield in rice. Rice 9:64 https://doi.org/10.1186/s12284-016-0136-z
- Fujita D, Trijatmiko KR, Tagle AG, Sapasap MV,Koide Y,Sasaki K, Tsakirpaloglou N, Gannaban RB,Nishimura T, Yanagihara S, Fukuta Y, Koshiba T, Slamet-Loedin IH, Ishimaru T and Kobayashi N (2013) NAL1 allele from a rice landrace greatly increases yield in modern indica cultivars. Proceedings of the National Academy of Sciences 110 (51):20431-0436.



- Fukai S and Cooper M (2001) Development of drought resistant cultivars for rainfed lowland rice experience from Northeast Thailand and surrounding areas. In: Proceedings of International Conference on the Impact of Agricultural Research for Development in Southeast Asia, CARDI, Phnom Penh. Cambodia. 24–26 October 2000, pp. 185–194.
- Fukao T and Xiong L (2013) Genetic mechanisms conferring adaptation to submergence and drought in rice: simple or complex? Current Opion in Plant Biology 16(2):196-204. doi: 10.1016/j.pbi.2013.02.003.
- Guo L, Zhu L, Xu Y, Zeng D, Wu P and Qian Q (2004) QTL analysis of seed dormancy in rice (*Oryza sativa* L.). Euphytica 140(3):155-62.
- Hattori Y, Nagai K, Furukawa S, Song XJ, Kawano R, Sakakibara H, Wu J, Matsumoto T, Yoshimura A, Kitano H, Matsuoka M, Mori H and Ashikari M (2009) The ethylene response factors SNORKEL1 and SNORKEL2 allow rice to adapt to deepwater. Nature 460:1026–1030.
- Huang X, Qian Q, Liu Z, Sun H, He S, Luo D, Xia G, Chu C, Li J and Fu X (2009) Natural variation at the *DEP1* locus enhances grain yield in rice. Nature Genetics 41:494–497.
- Ishimaru K, Hirotsu N, Madoka Y, Murakami N, Hara N, Onodera H, Kashiwagi T, Ujiie K, Shimizu B, Onishi A, Miyagawa H and Katoh E (2013) Loss of function of the IAA-glucose hydrolase gene TGW6 enhances rice grain weight and increases yield. Nature Genetics 45:707–711.
- Ishimaru K, Togawa E, Ookawa T, Kashiwagi T, Madoka Y and Hirotsu N (2008) New target for rice lodging resistance and its effect in a typhoon. Planta 227(3):601-9.
- Jiao Y, Wang Y, Xue D, Wang J, Yan M, Liu G, Dong G, Zeng D, Lu Z, Zhu X, Qian Q and Li J (2010) Regulation of OsSPL14 by OsmiR156 defines ideal plant architecture in rice. Nature Genetics 42(6):541-544. doi: 10.1038/ng.591.
- Kashiwagi T (2014) Identification of quantitative trait loci for resistance to bending-type lodging in rice (*Oryza sativa* L.). Euphytica 198:353–367.
- Kashiwagi T and Ishimaru K (2004) Identification and functional analysis of a locus for improvement of lodging resistance in rice. Plant Physiology 134:676–683.
- Kashiwagi T, Munakata J and Ishimaru K (2016) Functional analysis of the lodging resistance QTL BSUC11. Euphytica 210(2):233-43.
- Khush GS, Mackill DJ and Sidhu GS (1989) Breeding rice for resistance to bacterial leaf blight. In: IRRI (ed) Bacterial blight of rice. IRRI, Manila, Philippines, pp 207–217.
- Kim HB, Cho JI, Ryoo N, Shin DH, Park YI, Hwang YS, Lee SK, An G and Jeon JS (2016) Role of rice cytosolic hexokinase OsHXK7 in sugar signaling and metabolism. Journal of Integrative Plant Biology 58(2):127-35. doi: 10.1111/jipb.12366.
- Kitomi Y, Kanno N, Kawai S, Mizubayashi T, Fukuoka S and Uga Y (2015) QTLs underlying natural variation of root growth angle among rice cultivars with the same functional allele of deeper rooting 1. Rice 8:16. https://doi.org/10.1186/s12284-015-0049-2
- Kretzschmar T, Pelayo MAF, Trijatmiko KR, Gabunada LFM, Alam R, Jimenez R, Mendioro MS, Slamet-Loedin IH, Sreenivasulu N, Bailey-Serres J, Ismail AM, Mackill DJ and Septiningsih EM (2015) A trehalose-6-phosphate phosphatase enhances anaerobic germination tolerance in rice. Nature Plants 15124.doi: 10.1038/nplants.2015.124
- Lee GA, Jeon YA, Lee HS, Hyun DY, Lee JR, Lee MC, Lee SY, Ma KH and Koh HJ (2017) New Genetic Loci Associated with Pre-harvest Sprouting and Its Evaluation Based on the Model Equation in Rice. Frontiers in Plant Science 8:1393.
- Li Y, Fan C, Xing Y, Jiang Y, Luo L, Sun L, Shao D, Xu C, Li X, Xiao J, He Y and Zhang Q (2011) Natural variation in GS5 plays an important role in regulating grain size and yield in rice. Nature Genetics 43:1266–1269.



- Magwa RA, Zhao H and Xing Y (2016) Genome-wide association mapping revealed a diverse genetic basis of seed dormancy across subpopulations in rice (*Oryza sativa* L.). BMC Genetics 17(1):28.
- Marzougui S, Sugimoto K, Yamanouchi U, Shimono M, Hoshino T, Hori K, Kobayashi M, Ishiyama K, Yano M (2012) Mapping and characterization of seed dormancy QTLs using chromosome segment substitution lines in rice. Theoretical and Applied Genetics 124(5):893-902.
- Mohanty SK, Panda RS, Mohapatra SL, Nanda A, Behera L, Jena M, Sahu RK, Sahu SC and Mohapatra T (2017) Identification of novel quantitative trait loci associated with brown planthopper resistance in the rice landrace Salkathi. Euphytica 213(2):38.
- Narsai R, Gouil Q, Secco D, Srivastava A, Karpievitch YV, Liew LC, Lister R, Lewsey MG and Whelan J (2017) Extensive transcriptomic and epigenomic remodelling occurs during *Arabidopsis thaliana* germination. Genome Biology 18:172. https://doi.org/10.1186/ s13059-017-1302-3.
- Narsai R, Edwards JM, Roberts T H, Whelan J, Joss GH and Atwell BJ (2015) Mechanisms of growth and patterns of gene expression in oxygen-deprived rice coleoptiles. Plant Journal 82:25–40. 10.1111/tpj.12786.
- Neeraja CN, Maghirang-Rodriguez R, Pamplona A, Heuer S, Collard B, Septiningsih E, Vergara G, Sanchez D, Xu K, Ismail A and Mackill DJ (2007) A marker-assisted backcross approach for developing submergence tolerant rice cultivars. Theoretical and Applied Genetics 115:767-776.
- Ookawa T, Hobo T, Yano M, Murata K, Ando T, Miura H, Asano K, Ochiai Y, Ikeda M, Nishitani R, Ebitani T (2010) New approach for rice improvement using a pleiotropic QTL gene for lodging resistance and yield. Nature Communication 1:132.
- Ookawa T, Inoue K, Matsuoka M, Ebitani T, Takarada T, Yamamoto T, Ueda T, Yokoyama T, Sugiyama C, Nakaba S and Funada R (2014) Increased lodging resistance in long-culm, lowlignin gh2 rice for improved feed and bioenergy production. Scientific reports 4:6567. doi: 10.1038/srep06567.
- Pradhan SK, Nayak DK, Pandit E, Behera L, Anandan A, Mukherjee AK, Lenka S and Barik DP (2016) Incorporation ofbacterial blight resistance genes into lowland rice cultivar through marker-assisted backcross breeding. Phytopathology http://dx.doi.org/10.1094/PHYTO-09-15-0226-R
- Pradhan SK, Nayak DK, Mohanty S, Behera L, Barik SR, Pandit E and Lenka S (2015) Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deepwater rice variety, Jalmagna. Rice DOI 10.1186/s12284-015-0051-8
- Rathi S, Baruah A, Chowdhury R and Sarma R (2011) QTL Analysis of seed dormancy in indigenous rice of Assam, India. Cereal Research Communication 39(1):137-46.
- Sasaki K, Kazama Y, Chae Y and Sato T (2013) Confirmation of Novel Quantitative Trait Loci for Seed Dormancy at Different Ripening Stages in Rice. Rice Science 20(3):207-212.
- Singh GP, Srivastava MK, Singh RM and Singh RV (1977) Variation in quantitative and *Xanthomonas* oryzae. Plant Disease Report 57:537–541
- Song XJ, Huang W, Shi M, Zhu MZ and Lin HX (2007) A QTL for rice grain width and weight encodes a previously unknown RING-type E3 ubiquitin ligase. Nature Genetics 39: 623–630.
- Takeuchi Y, Lin SY, Sasaki T and Yano M (2003) Fine linkage mapping enables dissection of closely linked quantitative trait loci for seed dormancy and heading in rice. Theoretical and Applied Genetics 107(7):1174-80.



- Turner AG and Annamalai H (2012) Climate change and the South Asian summer monsoon. Nature Climate Change 2(8):587-95.
- UgaY, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N, Inoue H, Takehisa H, Motoyama R, NagamuraY, Wu J, Matsumoto T, Takai T, Okuno K andYano M (2013) Control of root system architecture by deeper rooting 1 increases rice yield under drought conditions. Nature Genetics 45(9):1097-102. doi: 10.1038/ng.2725. 45(9):1097-102.
- Vijayan J, Senapati S, Ray S, Chakravorty K, Molla KA, Basak N, Pradhan B, Yeasmin L, Chattopadhay K and Sarkar RK (2018) Transcriptomic and physiological studies identifies clues for germination stage oxygen deficiency tolerance in rice. Environmental and Experimental Botany 147:234-248.
- Wang L, Cheng J, Lai Y, Du W, Huang X, Wang Z and Zhang H (2014) Identification of QTLs with additive, epistatic and QTL× development interaction effects for seed dormancy in rice. Planta 239(2):411-20.
- Wang S, Wu K, Yuan Q, Liu X, Liu Z, Lin X, Zeng R, Zhu H, Dong G, Qian Q, Zhang G and Fu X (2012) Control of grain size, shape and quality by OsSPL16 in rice. Nature Genetics 44:950–954.
- Weng J, Gu S, Wan X, Gao H, Guo T, Su N, Lei C, Zhang X, Cheng Z, Guo X, Wang J, Jiang L, Zhai H and Wan J (2008) Isolation and initial characterization of GW5, a major QTL associated with rice grain width and weight. Cell Research 18:1199–1209.
- Wissuwa M, Wegner J, Ae N and Yano M (2002) Substitution mapping of *Pup1*: a major QTL increasing phosphorus uptake of rice from a phosphorus deficient soil. Theoretical and Applied Genetics 105: 890–897.
- Xu K, Xia X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC and Mackill DJ (2006) Sub1A is an ethylene response factor-like gene that confers submergence tolerance to rice. Nature 442:705–708.
- Xu K, Xu X, Ronald PCand Mackill DJ (2000) A high-resolution linkage map in the vicinity of the rice submergence tolerance locus Sub1. Molecular Genomics and Genetics 2000: 263:681– 689.
- Xue W, Xing Y, Weng X, Zhao Y, Tang W, Wang L, Zhou H, Yu S, Xu C, Li X and Zhang Q (2008) Natural variation in *Ghd7* is an important regulator of heading date and yield potential in rice. Nature Genetics 40:761–767 10.1038/ng.143
- Yadav S, Singh UM, Naik SM, Venkateshwarlu C, Ramayya PJ, Raman KA, Sandhu N and Kumar A (2017) Molecular Mapping of QTLs Associated with Lodging Resistance in Dry Direct-Seeded Rice (*Oryza sativa* L.). Frontier in Plant Science 8:1431.
- Yano K, Ookawa T, Aya K, Ochiai Y, Hirasawa T, Ebitani T, Takarada T, Yano M, Yamamoto T, Fukuoka S and Wu J (2015) Isolation of a novel lodging resistance QTL gene involved in strigolactone signaling and its pyramiding with a QTL gene involved in another mechanism. Molecular Plant 8(2):303-14.
- Zhou Y, XieY,Cai J, Liu C,Zhu H, Jiang R, Zhong Y, Zhang G, Tan B, Liu G, Fu X, Liu Z, Wang S, Zhang G and Zeng R (2017) Substitution mapping of QTLs controlling seed dormancy using single segment substitution lines derived from multiple cultivated rice donors in seven cropping seasons. Theoretical and Applied Genetics 130(6):1191-1205, PMID: 28283703.\*



## Genetic Improvement of Rice for Multiple Stress Tolerance in Unfavorable Rainfed Ecology

#### K Chattopadhyay, JN Reddy, SK Pradhan, SSC Patnaik, BC Marndi, P Swain, AK Nayak, A Anandan, K Chakraborty, RK Sarkar, LK Bose, JL Katara, C Parameswaram, AK Mukherjee, SD Mohapatra, A Poonam, SK Mishra and RR Korada

#### SUMMARY

Due to various abiotic stresses very low productivity of rice is noted from unfavourable rainfed lowland ecology, which accounts 18% of total rice growing area. Gradual change in climatic scenario including monsoon results in various abiotic stresses in this ecology frequently at various stages of crop growth in isolation or in combination. Coastal saline ecology is affected by salinity and excess water stresses. Varieties developed for this ecology through conventional and marker-assisted breeding are not significantly tolerant for either salinity or multiple abiotic stresses such as salinity and water logging at reproductive stage. Similarly for semi-deep and deep water ecology also requires biotic stress tolerance and tolerance to water logging and prolonged submergence. Anaerobic germination ability is also requisite in high yielding background for direct sowing in unfavourable lowland ecology. Validation of QTLs including those identified by National Rice Research Institute (NRRI), Cuttack for salt tolerance at reproductive stage is one of current research priorities. Conventional breeding for multiple stress (salinity and excess water) tolerance is being complemented by the efforts at NRRI, Cuttack to identify multiple stress tolerance QTLs and introgression in high yielding background suitable for coastal ecology. Research priorities for sustainable rice production in semi-deep and deep water ecology include identification of QTLs for anaerobic germination ability and prolonged submergence tolerance and pyramiding them along with genes for water logging and biotic stress tolerance. Improvement of productivity and sustainability of this ecology through our research efforts would definitely elevate the country's overall rice productivity and ensure social and economic security of people who depend on this ecology for their livelihood.

#### **1. INTRODUCTION**

Rainfed ecology can be divided under rainfed upland, favourable lowland and unfavourable lowland with flood prone and salinity affected areas. The low productivity of this ecology is mainly due to various abiotic stresses, which are found alone or in combination in the various crop growth stages. Therefore, enhancement of abiotic stresses tolerance can only able to improve the production, productivity and overall profitability of the system. Rice is sensitive to salinity at both seedling and reproductive stages. Although rice is sensitive to salt stress, it is a preferred crop in salt-affected coastal areas. Rice can withstand water-logging, and standing water



helps in diluting and leaching salts from surface soil (Ismail et al. 2008). Therefore, despite their low yields, many landraces are still preferred by farmers in coastal areas. The degree of salt tolerance varies widely in these landraces, and the variability offers an opportunity for varietal improvement for salt tolerance. On the backdrop of lack of significant improvement of salinity tolerance in rice through traditional breeding. marker assisted breeding using major QTL (Saltol) for seedling stage salt tolerance was initiated in India and worldwide. Some of the introgressed high yielding varieties were showing better survivability but that could not guarantee high yield when salt stress occurs at flowering stage. Mitigation of the salt stress effects on rice growth and yield has been tried through both management practices and introduction of tolerant varieties in the affected areas; but use of fresh water supply and other management practices alone in salt-affected areas has generally proven to be uneconomical and not feasible to implement on a large scale. Thus, genetic improvement of salt tolerance in rice is the most feasible and promising strategy to provide stable food production in such a stress-prone environment. In coastal areas rise in temperature, erratic rainfall along with penetration of salty water due to sealevel rise can change the micro environment in multifaceted manners (Wassmann et al. 2009). Multiple abiotic stresses are reality in coastal plains. Addressing multiple abiotic stress tolerance, as well as the consequences of one stress followed by another could develop knowledge related to adaptation of rice crop under variable climatic conditions.

Apart from coastal ecosystem, unfavourable rainfed ecosystem facing problem of prolonged submergence and water stagnation due to flood. Therefore, development of rice varieties with flood tolerance is prime requirement. *Sub1* gene has been identified, cloned and characterized from FR13A, an *indica* rice variety, which gives 14 days (two weeks) tolerance to submergence and it has also been transferred to number of rice varieties through marker assisted introgression. But due to climate change, lowland ecology is facing problems of prolonged submergence, which is often more than 15 days. Therefore, cultivar for such situation is urgently required for sustainable production. On the other hand, early direct sowing in semi-deep water logged situation before onset of monsoon is generally practiced. Moreover, sowing seeds in standing water is an effective means of weed control and direct seeded rice in rainfed ecology is getting popularity due to reduced cultivation cost. Therefore, anaerobic germination ability in rainfed unfavorable ecology in high yielding cultivars is highly needed.

This chapter describes the research problems related to the coastal saline, waterlogging and deep water ecosystems in the backdrop of changing climatic condition and various basic, strategic and applied research carried out at international, national and NRRI level. This also deals with the future strategies and way forward on these issues.

### 2. PROBLEMS OF UNFAVOURABLE RAINFED ECOLOGY

Traditional approaches for introducing improved rice varieties to farmers have demonstrated significant impact in favourable ecosystems. But limited success was achieved in unfavourable ecosystems, affected by various abiotic stresses such as



salinity, submergence, water logging and even drought in some cases. Through traditional breeding many varieties with high yield potential such as CR Dhan 403, CR Dhan 405, Panvel 3, Bhutnath, etc. have been developed for coastal saline areas. But none of them is tolerant to high salinity stress (>6 dS m<sup>-1</sup>), especially at flowering stage. The major reasons are relatively more complexity and genotype x environment interaction, indicators of physiological traits and well accepted screening protocol for salinity tolerance at flowering stage. Therefore, robust QTLs and markers are also unavailable for marker assisted selection for this trait. Due to lack of physiological traits controlling salinity tolerance at flowering stage and simplicity in screening protocol, any major QTL is yet to be identified and used in marker assisted selection for incorporating tolerance at reproductive stage.

Coastal saline areas are normally mono-cropped with rice grown during the *Kharif* season. Agricultural productivity is low and unstable due to the frequent occurrence of abiotic stresses. During our widespread survey and demonstrations of climate resilient rice varieties in coastal region we felt that salinity and stagnant flooding in combination affecting the rice production in greater extent (Chattopadhyay et al. 2016). Due to this reason still farmers grow local land races tolerant to both salinity and stagnant flooding. Flooding with saline water is a common problem. Intrusion of sea water displaces millions of people from coastal plains and causes the direct threat of the economic security of the poor and marginal people reside along the coastal belts (Wassmann et al. 2009). Development of rice tolerant to multiple abiotic stresses especially salinity and stagnant flooding could improve the productivity and sustainability of the coastal ecosystem.

Deep and semi-deep waterlogged area is another unfavorable lowland ecosystem. Problems associated with this ecosystem are as follows.

- Several rainfed rice areas are at risk of flooding, a stress that decreases yield substantially (Ismail et al. 2013). Poor crop establishment due to deep submergence and water logging at the early stage of the crop growth is predominant. Prolonged submergence/waterlogging suppresses tillering and increases mortality. Water logging and poor drainage also leads to accumulation of toxic substance causing problems such as iron toxicity and sulphide injury.
- Incidence of pests such as stem borer, gall midge, cutworm, GLH and leaf folder and diseases such as bacterial blight, sheath blight sheath rot, tungro and false smut along with abiotic stresses are also frequent in deep and semi-deep water logged areas.
- Submergence tolerance (*Sub1*) has been incorporated in many high yielding varieties adapted to rainfed lowland ecology (Singh et al. 2016). However, additional genes are needed for imparting flood tolerance for 21 days (3 weeks) or more because of regular flooding in India and south-east Asia due to climate change. Better genotypes with submergence tolerance at early and late vegetative stages for more than 21 days are not available.

124

• Direct seeding is a traditional system of sowing under rainfed ecosystem and it's getting popular in irrigated areas, as the decrease in labor source and increase in labor charges. This system of sowing helps in reducing cultivation charges, a week of crop duration and maintains the physical soil structure for successive cropping. The stagnation of water in unleveled fields after irrigation or copious rainfall immediately after sowing leads to poor germination. Poor establishment and uneven growth pattern due to the difference in germination affects timely intercultural operation (weed/fertilizer, etc), that leads to poor yield. Flash flooding during sowing to harvesting is the state of problem widespread across the south to Eastern states of the country (Anandan et al. 2012). The activity of  $\alpha$ -amylase determines the germination under water enhancing stress tolerance. No popular high yielding rice varieties possess this mechanism to germinate under anoxia. Traditional landraces have genes for this trait, but they are tall lodging type with red pericarp and susceptible to diseases and very poor phenotypic performance.

## 3. STATUS OF RESEARCH

During initial exposure to salinity, plants experience water stress, which in turn reduces shoot growth. As time of exposure to salinity is prolonged, plants experience ionic stress, which leads to leaf tip drying, premature senescence of leaves and mortality of plants. As salt stress is cumulative, injury symptoms increase with time. So, restricting the movements of toxic ions such as Na<sup>+</sup> or Cl<sup>-</sup> to growing meristematic tissues and young photosysnthetic organs are vital for survival. The procedure of screening rice genotypes for salt tolerance at seedling stage has been well established and validated through number of experiments. But due to long growing period of rice plants and complexity in measuring tolerance level, no protocol has been found ideal for high throughput screening till date. A salt tolerant (at seedling stage) cultivar, Pokkali has Na<sup>+</sup> exclusion mechanism and thereby maintains low Na<sup>+</sup>: K<sup>+</sup> ratio in shoot and new leaf. The Saltol QTL in rice was identified by employing a RIL population between the tolerant landrace Pokkali and the highly sensitive IR 29. Among more than 100 identified QTLs, this is the major QTL for seedling stage salt tolerance contributing 43% of variation for seedling shoot Na<sup>+</sup>- K<sup>+</sup> ratio. A plasma membrane transporter that regulates partitioning of Na<sup>+</sup> between roots and shoots, OsHKT1;5 was identified as one of the causative gene located inside the Saltol-QTL, which was fined mapped and cloned (Ren et al. 2005). Salinity tolerance in most of the Indica genotypes is generally correlated with low Na+ ions in shoot and OsHKT1;5 is found more active in those tolerant genotypes. But some wild rice accessions of O. glaberrima could exclude Na<sup>+</sup> from shoots using a mechanism independent of OsHKT1;5 (Platten et al. 2013). Introgression lines derived from O. rufipogon  $\times$  O. sativa cross revealed 15 QTLs for salinity tolerance, 13 of them derived from the tolerant O. rufipogon parent (Tian et al. 2011). A genotype with seedling stage salinity tolerance may not be tolerant at reproductive stage as well. For salt tolerance at reproductive stage, 16 QTLs for pollen fertility, Na<sup>+</sup> concentration, Na-K ratio in flag leaf in chromosome 1, 7, 8, 10 (Hossain et al. 2015) were identified. But none of them was validated.





Rice is unique compared to any other cereals to adapt with water stagnation. *Sub1* is the major QTL associated with submergence tolerance in rice. The locus was mapped to chromosome 9, and is composed of a cluster of ethylene response factors (ERF) genes located *in tandem*, named *SUB1A*, *SUB1B* and *SUB1C*. The tolerant *SUB1A-1* allele is derived from the *aus* subgroup of *indica* rice (Xu et al. 2006). Niroula et al. (2012) tested 109 accessions of cultivated and wild relatives, including 12 species, for submergence tolerance, and found *O. rufipogon* and *O. nivara* tolerant accessions that carry the *SUB1A-1* allele, showing that *SUB1* locus architecture determines submergence tolerance in these species, as in *O. sativa*. Rice plants adapt to very deep water stagnation (> 1 m deep) through greater elongation of stems by the action of the gene 'Snorkel' 1 and 2 (Hattori et al. 2009). On the other hand, five putative QTLs of qAG-1-2, qAG-3-1, qAG-7-2, qAG-9-1 and qAG-9-2 elucidating 17.9 to 33.5% of the PV from KhaoHlan On has been reported by Angaji et al. (2010) for anaerobic germination. Later six QTLs on chromosomes 2, 5, 6 and 7 from the landrace Ma-Zhan Red was identified by Septiningsih et al. (2013).

Traditionally cultivated local rice varieties in coastal area have tolerance to salinity and submergence but are low yielding. Some of the widely used varieties are: Vikas, Korgut, Sathi, Pichaneelu, Kuthiru, Kalundai samba, Bhurarata, Kalarata, Karekagga, Pokkali, Chettivirippu, Bhaluki, Rupsal, Nona Bokra, Kamini, Talmugur, Patnai, Getu, etc. These diverse traditional rice varieties are precious genetic resources that provide ecological balance and their conservation is crucial for future food security. Using these germplasm, many high yielding varieties such as CST1-7, Bhutnath, Panvel-3, CSR36, etc. have been developed. Sub1 gene is attempted to transfer into popular lowland rice varieties such as Bahadur, Ranjit, Varshadhan and Savitri. Similarly, Saltol-*QTL* for salt tolerance at seedling stage is being transferred into varieties, namely, ADT 45, Savitri, Gayatri, MTU 1010, PR 114, Pusa 44 and Sarjoo 52 (Singh et al. 2016). CSR 27 (Pandit et al. 2010) was identified as tolerant variety for salt tolerance at flowering stage. Number of QTLs for salt tolerance at flowering stage were identified, but none of them were found reproducible. Due to population specificity and limited scope of using diverse tolerant germplasm in bi-parental mapping, genome wide association mapping exploiting large scale single nucleotide polymorphism have been practiced to capture natural variations in loci and allelic variations in candidate genes for complex abiotic stresses such as salinity. Using custom-designed array based on 6000 SNPs, Kumar et al. (2015) identified 20 loci associated with Na-K homeostasis. They found Saltol as the major salt tolerance QTL not only for seedling stage, but also for reproductive stage in relation to Na<sup>+</sup>-K<sup>+</sup> ratio in leaves. New QTLs were also found on chromosomes 4, 6 and 7.

At NRRI, considerable research has been carried out on many aspects of crop improvement for unfavourable rainfed ecology.

# 3.1. Diversity of Saltol- QTL region and detection of Saltol introgression lines

Landraces from Sundarban region were found diverse in respect of salt tolerance. Salt tolerant cultivars from this area such as Kamini, Talmugur, etc. had allelic difference





Fig. 1. High yielding salinity tolerant line (IET 23400) from the cross Annapurna/ FL 478 carrying *Saltol* QTL.

from the widely used *Saltol*- introgression line, FL 478 in the *Saltol* - QTL region. From IR64/FL478 cross, eight  $F_8$  tolerant and moderately tolerant lines at seedling stage (at EC= 12 dSm<sup>-1</sup>) along with their parents (FL 478 and IR 64) were subjected to analysis for validation of the microsatellite markers in the *Saltol* QTL region. Four primers (RM10694, RM8094, AP3206, RM493) in *Saltol* region were found polymorphic between FL 478 and IR64. FL478 specific marker alleles for different loci situated from 11 Mb to 12.4 Mb region

in chromosome 1 was found in all the lines tested in homozygous condition. These tolerant and moderately tolerant lines sharing a common segment from the donor FL 478 might carry the *Saltol* QTL in this region. Thirty seven tolerant and moderately-tolerant (SES=3-5)  $F_7$  lines (with 3-5 t ha<sup>-1</sup> yielding ability (Fig. 1) under coastal saline situation at dry season) derived from the Annapurna × FL478 cross. These lines are sharing a common segment from the donor FL 478 might carry the *Saltol* QTL (Fig. 2) either in homozygous or heterozygous condition (Chattopadhyay et al. 2014).

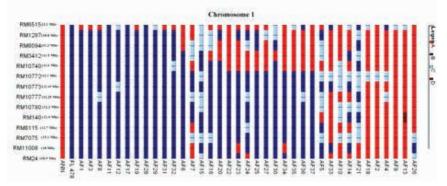


Fig. 2. Graphical genotyping of salt tolerant lines derived from Annapurna/FL 478 carrying Saltol region in chromosome 1.

#### 3.2. Parental combination for improvement of salt tolerance

Unlike Pokkali, Rahspunjar was efficient in maintaining higher level of K<sup>+</sup> despite high Na<sup>+</sup> influx in shoot and located distant from Pokkali in 3-D plot on SSR data. Morpho-physiological difference and the highest allelic difference between SR 26B and Pokkali in the *Saltol* QTL region was supported by non-significant association between *Saltol* marker RM 10745, RM 3412 with tolerance phenotype. Swarna *Sub1* × Rahspunjar and Savitri × SR 26B produced more transgression segregants for tolerance and were found ideal combination (Chattopadhyay et al. 2015).



#### 3.3. Standardization of protocol, identification of donors and understanding the reproductive stage salinity tolerance in rice

Chattopadhyay et al. (2017a) standardized a protocol where setup was established with a piezometer placed in a perforated pot for continuous monitoring of soil EC and pH. Further, fertilized soil was partially substituted by gravels for stabilization and maintaining the uniformity of soil EC in pots without hindering its buffering capacity. The protocol having modified medium (soil:stone 4:1) at 8 dSm<sup>-1</sup> salinity level was validated using seven different genotypes having differential salt sensitivity. Based on this new medium, important selection traits such as high stability index for plant yield, harvest index and number of grains/panicle and also high K<sup>+</sup> concentration and low Na<sup>+</sup>-K<sup>+</sup> ratio in flag leaf at grain filling stage were validated and employed in the evaluation of a mapping population. The method was found remarkably efficient for easy maintenance of desired level of soil salinity for identification of genotypes tolerant to salinity at reproductive stage and evaluation of mapping population.

We have also identified tolerant germplasm with QTL linked markers for salt tolerance at flowering stage using 8 dS m<sup>-1</sup> NaCl water (Chattopadhyay et al. 2013). Donors for reproductive stage tolerance viz. AC41585, AC39394 (Chattopadhyay et al. 2013), were validated with this method. Results showed that visual scoring of stress symptom and/or SPAD reading may not correspond to the stress effect. Better phenotyping technique such as chlorophyll fluorescence imaging can show clear cut differences between salt treated and untreated rice plants at reproductive stage. Gene expression analysis revealed that salt tolerant Pokkali (AC 415858) genotype showed better K<sup>+</sup>-retention and Na<sup>+</sup>-exclusion strategies coupled with maintenance of better membrane potential (both plasma membrane and vacuolar) by induction of ATPases and PPases activities in flag leaf (NRRI Annual report 2016-17).

Many QTLs have been identified for salt tolerance at seedling and reproductive stages. But none of them could be validated, fine mapped and cloned for using in salt tolerance breeding programme (Chattopadhyay et al. 2013). At NRRI, 180 backcross derived lines (BC<sub>3</sub>F<sub>3.5</sub>) from salt tolerant donor AC41585 and recurrent parent IR 64 were subjected to phenotype in saline (EC= 8 dS m<sup>-1</sup>) and non-saline environments in 2014 and 2015. Normal distribution with small skewness values was found for all these significant yield attributing traits. Polymorphic 121 SSR, hyper variable-SSR and gene based primers were used and data were analysed via inclusive composite in-terval mapping and two dimensional scaling using QTL IciMapping v4.0.6. Map covered a genetic distance of 1235.53 cM. Two main effect additive QTLs for DEG-S on chromosome 2 and 4 and five additive QTLs for stress susceptibility index for sterility (SSI-STE) on chromosome 2, 3, 4 and 11 with 17-42% phenotypic variance were found common under salinity stress over the years. A main effect QTL with pleiotropic effect for SSI-STE and DEG-S in 11-15 cM region on chromosome 2 was found in marker interval HvSSR02-50 - RM13263 in 2015. Single marker analysis revealed that over the years two markers RM17016 at 64.84cM position on chromosome 1 and



HvSSR06-63 at 77.41 cM position on chromosome 6 were associated with STE-S. Functional genes (Os01g38980.1, Os06g45940.1, Os02g31910.1, Os02g33490.1) located inside or just adjacent (3 cM) to QTLs detected through composite interval mapping and single marker analysis. Functional validation is required for detection of their possible role in salt tolerance at flowering stage (Chattopadhyay et al. 2017b).

#### 3.4. Rice varieties for coastal saline areas

Lunishree, the first high yielding variety for coastal saline area was developed by NRRI, Cuttack. In recent years (for the last six years) high yielding varieties such as Luna Sampad, Luna Suvarna and Luna Barial has been developed by ICAR-NRRI for wet season and Luna Sankhi (Fig. 3) has been developed by ICAR-NRRI in collaboration with IRRI, Philippines for dry season cultivation in coastal saline areas. CR Dhan 402 (Luna Sampad, IET 19470) and CR Dhan 403 (Luna Suvarna, IET 18697) were developed at the National Rice Research Institute (CRRI), Cuttack and released by the Odisha State Sub-Committee on Crop Standards (State Varietal Release Committee) in 2010. They were found promising in testing under the All India Coordinated Rice Improvement Programme (AICRIP), participatory varietal selection (PVS) and other on-farm trials conducted in rainfed coastal saline areas of Odisha. The average grain



Fig. 3. Field view of Luna Sankhi (CR Dhan 405) developed for coastal saline areas in dry season in Odisha.

yield of Luna Suvarna recorded over the four years of testing in Jagatsinghpur, Kendrapara and Puri districts of Odisha was 4.6 t ha<sup>-1</sup>. It had shown an average 17% yield superiority over the national check CST 7-1 in the All India Coordinated trial. The variety was also found promising in Gosaba, Basanti and Sandeshkhali blocks in the Sundarban area of West Bengal. Luna Sampad also out yielded CST 7-1, the national check and Lunishree in all India Coordinated trial. It is well accepted by farmers of

Basudevpur of Bhadrak, Marshaghai of Kendrapara and Puri districts of Odisha with average yield with 3.6 to 4.2 t ha<sup>-1</sup>. Another rice variety, Luna Barial (CR Dhan 406, IET 19472), developed at the NRRI, Cuttack was released by the Odisha State Sub-Committee on Crop Standards in 2012. This variety was ranked first (3908 kg/ha) in eastern zone in the All India Coordinated trial. Under multilocation trials, it has shown yield superiority with 3.7-4.5 t ha<sup>-1</sup> over national and local checks in Ganjam, Cuttack, Balasore and Khurda districts of Odisha. All the three varieties can be grown along the coastal belt of eastern India with medium salinity stress (EC 5-7 dS m<sup>-1</sup>). The parentage and important features of these varieties are listed in Table 1.



Sl No	Variety	Ecology	Parentage	Year of release	Released by CVRC/ SVRC	Duration (PS)	Reaction to biotic and abiotic stresses
1	Utkalprabha	Medium/ Semi-deep	Waikyaku/ CR 1014	1983	Odisha	155	MR or field tolerance to major pest and diseases
2	CR 1014	Medium/ Semi-deep	T 90/Urang Urangan	1988	Odisha	160	MR to Sh. B. MR or field tolerance to all pest and diseases
3	Gayatri	Medium/ Semi-deep	Pankaj/ Jagannath	1988	Odisha, West Bengal, Bihar	160	MR to Sh. B. MR or field tolerance to all pest and diseases
4	Kalashree	Medium deep	CR 151-79/ CR 1014	1988	Odisha	160	Tolerant to Blast and GM
5	Panidhan	Medium/ Semi-deep	CR 151-79/ CR 1014	1988	Odisha	180	Tolerant to Blast and GM
6	Tulasi	Medium/ Semi-deep	CR 151-79/ CR 1014	1988	Odisha	170	Field tolerance to major pests and diseases
7	Sarala	Medium/ Semi-deep		2000	Odisha	150	Intermediate, non- lodging, Photosensi- tive
8	Durga	Medium/ Semi-deep		2000	Odisha	155	Resistant to RTD and suitable for late planting
9	Varshadhan	Medium/ Semi-deep	IR 31432- 8-3-2/IR 31406-3-3- 3-1//IR 26940-3- 3-3-1	2006	Odisha	160	Non lodging and suitable for water logging situation
10	Hanseswari (CR Dhan 70)	Medium/ Semi-deep		2008	Odisha	150	MR- Blast, Sh B, Tol-False Smut, RTV
11	CR Dhan 501	Medium/ Semi-deep		2010	UP, Assam	152	R- Neck blast
12	CR Dhan 500	Deep water	Ravana / Mahsuri	2011	Odisha, UP	9 160	MR to leaf blast, neck blast, brown spot, gall midge biotype 1&5, stem borer dead heart and white-ear head damageand leaf folder attack
							<i>a</i> 1

# Table 1. High yielding rice varieties released by NRRI, Cuttack for semi-deep water logged, deep water and coastal saline ecologies.

Contd....



					Released		
				Year of			Reaction to biotic
	o Variety	Ecology	Parentage	release	SVRC	(PS)	and abiotic stresses
13	Jayanti (CR Dhan 502)	Deep water	Samson Polo/ Jalanidhi	2012	Odisha	160	MR- leaf blast, neck blast, sheath bight, sheath rot, rice tungro virus and gall midge biotype1 R- stem borer , leaf folder, rice thrips, and whorl maggot.
14	Jalamani (CR Dhan 503)	Deep water	Panikekoa/ Ambika	2012	Odisha	160	MR- leaf folder, green leaf hopper, leaf blast, neck blast, brown spot, gall midge, dead heartand stem borer
15	CR Dhan 505	Deep water	CRLC 899/ Ac.38606	201400	disha and As	ssam162	MR-blast, neck blast, sheath rot, sheath blight and rice tungro virus, stem borer, leaf folder, whorl maggot, submergence tolerance, elongation ability
16	CR Dhan 506	Semideep	CRLC 899/ Warda2	2017	Assam, Andhra Pradesh & Karnataka		-
17	Prasant (CR Dhan 507)	Semideep	Gayatri/ Sudhir// Varshadhan	2016	Odisha	160-165	MR-NBL, BS, ShB, ShR,SB, LF
18	CR Dhan 508	Deep water	CRLC 899/ Warda2	2017	Odisha, West Bengal, Assam	187	MR-sheath blight, brown spot and sheath rot
19	Lunishree	Coastal saline	Nonasail Gamma Irradiated Mutant	1992	Odisha/ CVRC	145	Tolerant to coastal salinity
20	Luna Sampad (CR Dhan 402)	Coastal saline	Mahsuri / Chakrakanda	2010	Odisha	140	Tolerant to coastal salinity
21	Luna Suvarna (CR Dhan 403)		Mahsuri / Ormundakan	2010	Odisha	150	Tolerant to coastal salinity
22	Luna Barial (CR Dhan 406)		Jaya / Lunishree	2012	Odisha	155	Tolerant to coastal salinity
23	Luna Sankhi Co (CR Dhan 405)		IR31142-14- 1-1-3-2/ IR71350	2012	Odisha	120	Tolerant to coastal salinity



# 3.5. Enhancing excess water tolerance and biotic stress tolerance for unfavourable rainfed lowland ecology

By screening of thousands of traditional landraces at ICAR-NRRI, several tolerant sources form different genetic background was identified and several rice varieties suitable for deep and semi-deep water ecosystem have been developed. Varshadhan (Fig. 4), Sarala, Gayatri, CR Dhan 500 and CR Dhan 505 are popular among them (Table 1). Molecular marker integrated backcross breeding program has been employed to transfer three major BB resistance genes (Xa21, xa13 and xa5) into Jalmagna variety. The three major BB resistance genes pyramided lines exhibited high level of resistance and provided durable resistance under deep water situation (Pradhan et al. 2015). The ICAR-NRRI, Cuttack identified a genotype, AC 20431B, which gives submergence tolerance up to 21 days. A mapping population was developed by crossing of Swarna-Sub1 and AC 20431B for mapping and identification of novel genes responsible for submergence tolerance other than *Sub1* gene. An attempt was also made for identification of linked marker(s) for 21 days submergence tolerance. The well characterized *Sub1* gene gives submergence tolerance for 14 days (2 weeks). Therefore,

Swarna-Sub1 was used as one of the parent with AC 20431B (donor parent) for mapping and identification of 21 davs submergence tolerance genes other than Sub1. A set of 568 F<sub>2</sub> plants were submerged for 21 days. Based on selective genotyping results, a marker RM27322, located on chromosome 11 was found to be linked with 21 days submergence tolerance. This marker will be further validated by genotyping of fixed population (NRRI Annual Report 2017).



Fig. 4. Popular rice variety, Varshadhan in water logged condition at Sandeshkhali-1 block of Sundarban.

# 3.6. A transcriptomic study to understand the combined effect of waterlogging and salinity stress in rice

In coastal-saline belts rice often faces combined stresses of waterlogging and salinity during different phases of growth. To assess the physiological and metabolic changes in rice associated with waterlogging and salinity stresses, a transcriptome profiling was performed in two waterlogging tolerant rice genotypes, Varshadhan (salinity susceptible) and Rahspunjor (salinity tolerant). Transcriptome analysis in leaf sheath at reproductive stage revealed that in response to waterlogging stress a total of 1489 and 1028 genes were differentially expressed in Varshadhan and Rahspunjor, respectively. Interestingly, combined stress of waterlogging and salinity (WS) resulted in fewer numbers of differentially expressed genes (748 and 840 in Varshadhan and Rahspunjor, respectively) in both the genotypes. Although both the

studied genotypes were tolerant to waterlogging stress, but the transcriptome data primarily indicated existence of differential tolerance mechanisms in them. Varshadhan showed up-regulation of hormonal biosynthesis pathway genes (ethylene and gibberellic acid) and triggers NADPH oxidase activity pointing towards ethylene dependent aerenchyma formation, while Rahspunjor showed up-regulation of genes related plant growth (SPL 8, SPL 16 etc.) as stress induced response. The combined stress (WS) showed up-regulation of Ca<sup>2+</sup>-dependent signalling (Ca<sup>2+</sup>-ATPase, CAX etc.) in both the genotypes, but the induction was more pronounced in Rahspunjor. Changes in the expression level of key K<sup>+</sup>-transporters (up-regulation of HAK5 and down-regulation of AKT1) emphasized better K<sup>+</sup>-retention ability in Rahspunjor under salinity stress contributing towards its salt-tolerant behaviour as compared to Varshadhan (Chakraborty et al. 2017).

#### 3.7. Breeding for multiple abiotic stress tolerance for coastal ecology

Few elite breeding lines with salt and waterlogging tolerance were performing well in multilocational testing (Fig. 5).

 Salt tolerant lines, CR 2459-23-1-1-S-B1-2B-1 (Gayatri/Rahspunjar) (IET 25101) and CR 2839-1-S-10-B2-B-43-3B-1 (Swarna/FL 496) (IET 25078) were also performed

well in waterlogged situation with estimated grain yield of 4 t/ha. These lines were also promoted to AVT-1 in CSTVT trial.

- Salinity tolerant lines with Waterlogging tolerance were identified 2016-17
  - CR 2851-S-1-B-4-1-1-1-1 (Gayatri/SR 26B)- 160 days MS yield- 4403 kg ha<sup>-1</sup>
  - CR 2850-S-2B-12-1-1-2-1-1 (Gayatri/FL 496) 160 days-MS- 4616 kg ha<sup>-1</sup>



Fig. 5. IET 25078 (CR 2839-1-S-10-B2-B-43-3B-1) performed well in salinity and stagnant flooding affected Basanti block of Sundarban, West Bengal in 2017.

#### 3.8. Researches on agronomic practices for coastal ecosystem

On-farm trials were conducted in the Ersama block of Jagatsinghpur district (Odisha) using rice varieties were selected nutrient management practices were evaluated and the most promising options were validated in participatory farmer-managed trials during 2004-2007 at six to eight locations. In the shallow lowlands, the findings suggest that under both the shallow and intermediate lowlands, *Sesbania* for the wet season and *Azolla* biofertilizer for the dry season are promising organic nutrient sources that can improve soil quality and contribute to enhancing and sustaining crop productivity





in coastal areas. Among different integrated nutrient management practices, *Sesbania* green manuring (GM) for intermediate lowlands (0-50 cm water depth), *Sesbania* GM + prilled urea (PU) and *Sesbania* GM + *Azolla* for shallow lowlands (0-30 cm water depth) in the wet season, and *Azolla* + PU in the dry season were found to be promising (Singh et al. 2009).

Poor crop stand and low fertilizer inputs are the important causes of poor and unstable rice yields in coastal saline ecosystem with multiple stresses. Appropriate crop establishment and nutrient management technology options were validated through farmers' participatory on-farm trials. Use of robust aged (50-day old) seedlings raised with nursery fertilization and closer planting (15x10 cm) in the wet season, and early planting (January 1<sup>st</sup> fortnight) in the dry season significantly improved the crop survivability and yield. However, substantial yield improvements (91% in wet and 75% in dry season) could be achieved by combining salt-tolerant varieties with improved crop management (Saha et al. 2008).

Studies on water management for dry season rice indicated that marginally-saline (EC 2.4-3.1 dS m<sup>-1</sup>) water could be used safely for two weeks during the vegetative stage under high salinity condition. Providing fresh water irrigation 2 days after disappearance of standing water during the vegetative stage produced as much yield as continuous ponding. These approaches would help in substantial saving of precious fresh water and expanding the cropping area, leading to enhanced land and water productivity. For non-rice crops, the highest yields of sunflower and groundnut were obtained with 4 cm irrigation at 15 day intervals.

## 4. KNOWLEDGE GAPS

The following questions are to be addressed in the future research programme for improvement of abiotic stress tolerance in unfavourable rainfed ecology.

- What is the robust QTL for salt tolerance at reproductive stage in rice?
- Is it possible to identify multiple stress tolerance QTLs in rice?
- Can we incorporate both salinity and waterlogging tolerance to a high yielding background in coastal ecology through traditional and marker-assisted breeding?
- What is the robust QTL for anaerobic germination ability required for direct sowing in lowland rainfed ecology?
- Can we utilize source for improvement of tolerance for prolonged submergence for more than 2 weeks?
- Can we combine biotic stress tolerance (Bacterial blight and Stem borer) with abiotic stress tolerance in unfavourable rainfed system?

134



# 5. RESEARCH AND DEVELOPMENT NEEDS AND RESEARCH PRIORITIES

# 5.1. Breeding for desirable plant characters of rice for water logged and deep water ecologies

- Medium/semi-deep water logged lowlands: Desirable plant characters for this ecology include 115-130 cm plant height, stiff culm, erect leaves, low to moderate tillering ability, high N-use efficiency at low N level, early seedling vigour, drought tolerance at seedling stage, prolonged submergence (beyond 15 days) tolerance with less elongation and without culm elongation, photoperiod-sensitive, thermo-insensitive, heavy panicle weight type and strong seed dormancy.
- Deep water ecology: Desirable plant characters for this ecology include 130-160 cm plant height, stiff culm, erect leaves, low tillering ability, high N- use efficiency at low N level, early seedling vigour, drought tolerance at seedling stage, submergence tolerance with leaf sheath and culm elongation, photoperiod-sensitive, thermo-insensitive, heavy panicle weight type, strong seed dormancy and kneeing ability.

### 5.2. Breeding scheme for water logging condition

■ For multiple abiotic stress tolerance breeding for areas where submergence at early stage of crop growth was followed by the stagnant flooding at the later stage was frequently occurred, F<sub>2</sub>-F<sub>3</sub> breeding lines are screened for submergence followed by screening for pest-diseases and evaluation for stagnant flooding tolerance. The tolerant check for screening for submergence tolerant is Swarna Sub1 and for stagnant water are IRRI 154 and Khoda.

### 5.3. Breeding for desirable plant architecture for coastal saline ecology

- Coastal salinity at wet season: Desirable plant characters for this ecology include 120-130 cm plant height, more than 145 days duration, stiff culm, erect leaves, moderate tillering ability, heavy panicle weight, early vigour, salinity tolerant at seedling and reproductive stages, submergence and/ or water logging tolerance with minimum elongation, photoperiod sensitive and strong seed dormancy.
- Coastal salinity at dry season: Desirable plant characters for this ecology include 100-110 cm plant height, 100-110 days duration, stiff culm, erect leaves, moderate to high tillering ability, early vigour, tolerant to salinity at both seedling and reproductive stages.

### 5.4. Breeding scheme for coastal saline condition

Wet season in eastern coastal area is affected not only by salinity but also by submergence at vegetative stage and water logging at different crop growth stages. Standardization of multiple stress tolerance breeding strategy and management practices to be given priority. The suitable breeding scheme for multiple abiotic stress tolerance in coastal saline areas in wet season is presented below with a schematic diagram (Fig. 6).





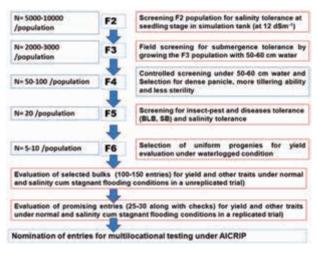


Fig. 6. Breeding scheme for multiple abiotic stress tolerance in coastal saline areas in wet season

## 5.5. Use of wide genetic base in rice improvement

Evaluation of wild accession can open up the possibility of getting better tolerance for abiotic (salinity and water logging) and biotic stress (stem borer, BB, etc.) required in unfavourable rainfed lowland ecology. Effort is needed to utilize these tolerance sources in developing agronomically superior elite rice lines. Introduction of wider genetic base in pre-breeding lines and identification of new QTLs associated with multiple abiotic stresses would help in developing more robust varieties for this unfavourable ecology.

# 5.6. Molecular breeding approaches for improvement of rice for unfavourable lowland ecology

- Saltol-QTL explained 46% variation for Na<sup>+</sup>-K<sup>+</sup> homeostasis leading to salt tolerance at seedling stage. QTLs other than Saltol to be identified and pyramided in tolerant lines along with Saltol QTL.
- Lot of scope for identification of better donors, their physiology and gene expression study in relation to salt tolerance at flowering stage. QTLs for reproductive stage salt tolerance are to be validated.
- Pyramiding of genes/QTLs for tolerant to submergence (*Sub1*), salinity (*Saltol*) and water logging in popular rice varieties of coastal saline areas are required.
- Pyramiding of genes/QTLs for anaerobic germination ability, tolerance to excess water submergence during germination and vegetative stages for getting successful crop at direct seeded rainfed lowland areas.
- Promising QTLs can be introgressed into popular rice varieties through MAB approach to improve their performance under flash flooded condition. If



pyramiding of different tolerant mechanism together into a single background would be beneficial to get tolerance under different microclimatic condition.

## 6. WAY FORWARD

The climate resilient varieties for rainfed unfavourable ecosystem should have multiple stress tolerance. The orientation of research is in that direction. Identification of new sources of multiple abiotic stress tolerance and development of mapping populations (Swarna/Rahspunjar, Savitri/AC39416a) for identification of QTL for multiple abiotic stress tolerance (salinity and waterlogging) is in progress. The salinity and waterlogging tolerant germplasm from cultivated and wild (O. rufipogon and O. nivara) rice collection are also being used for development of elite pre-breeding lines  $(BC_{*}F_{*})$ . On the other hand, research is focused on the identification of the robust QTLs other than Sub1 for excess water tolerance at various stages of crop growth and pyramiding them along with genes of important biotic stress tolerance. The use of community participatory approaches in the design, validation and dissemination of technologies is required to address problems of rice cultivation in unfavourable ecology. It is also required to anticipate and address constraints to the widespread adoption of new salt and water logging tolerant varieties and evaluate additional crops adapted to unfavourable ecology. Further studies will be carried out in the ICAR-NRRI-Regional Coastal Rice Research Station, Naira on the effects of soil salinity changes in the coastal districts on spatial and temporal scale and their consequences of production on the economics of high yielding and salt tolerant genotypes of rice.

#### References

- Anandan A, Rajiv G, Ramarao A and Prakash M (2012) Internode elongation pattern and differential response of rice genotypes to varying levels of flood water. Functional Plant Biology 39: 137–145.
- Angaji SA, Septiningsih EM, Mackill DJ, Ismail AM (2010) QTLs Associated with Tolerance of Flooding During Germination in Rice (*Oryza sativa* L.). Euphytica 172:159–168, doi: 10.1007/s10681-009-0014-5.
- Chakraborty K, Ray S, Ray A, Vijayan J, Molla KA, Chattopadhyay K, Sarkar RK (2017) A transcriptomic study to understand the combined effect of waterlogging and salinity stress in rice. Presented in Inter Drought- V at ICRISAT, Hyderabad from 21-25 Feb, 2017, 198.
- Chattopadhyay K, Nath D, Das G, Mohanta R L, Marndi B C, Singh D P, Sarkar R K, Singh O N (2013) Phenotyping and QTL-linked marker-based genotyping of rice lines with varying level of salt tolerance at flowering stage. Indian Journal of Genetics and Plant Breeding 73(4): 434-437.
- Chattopadhyay K, Nath D, Mohanta RL, Bhattacharyya S, Marndi B C, Nayak A K, Singh D P, Sarkar RK and Singh O N (2014). Diversity and validation of microsatellite markers in *Saltol* QTL region in contrasting rice genotypes for salt tolerance at the early vegetative stage. Australian Journal of Crop Science 8 (3): 356-362.
- Chattopadhyay K, Nath D, Mohanta RL, Marndi BC, Singh DP, Singh ON (2015). Morphophysiological and molecular variability in salt-tolerant and susceptible popular cultivars and their derivatives at seedling stage and potential parental combinations inbreeding for salt-tolerance in rice. Cereal Research Communication 43(2): 236–248, doi: 10.1556/CRC.2014.00445.





- Chattopadhyay K, Gayen S, Mondal I, Mishra SK, Mukherjee A K, Marndi BC, Singh O N and Sarkar R K (2016). Impact of Climate Resilient Varieties on Rice Productivity and Ensuring Food Security in coastal area in eastern India. NRRI Research Bulletin no. 10, ICAR -National Rice Research Institute, Cuttack, Odisha- 753006, India, 64.
- Chattopadhyay K, Marndi BC, Nayak AK, Mohanty SK, Poonam A, Guru PK, Reddy JN, Mahata KR, Singh DP, Sarkar RK (2017a). Salinity tolerance. In Enhancing climate resilience in rice: abiotic stress tolerance and greenhouse gas mitigation. Nayak AK, Sarkar RK, Chattopadhyay K, Reddy JN, Lal B and Chatterjee D (Eds) ICAR-National Rice Research Institute, Cuttack, Odisha- 753006, 48-63.
- Chattopadhyay K, Mohanty S K, Marndi BC and Sarkar RK (2017b) Main, epistatic and additive x environment interaction QTLs of contributing traits for salt tolerance at flowering stage in rice. ICAR-NRRI New letter 38 (2): 13-14.
- Hattori Y, Nagai K, Furukawa S, Song XJ, Kawano R, Sakakibara H, Wu J, Matsumoto T, Yoshimura A, Kitano H, et al (2009) The ethylene response factors *SNORKEL1* and *SNORKEL2* allow rice to adapt to deep water. Nature 460:1026-1030.
- Hossain H, Rahman MA, Alam MS and Singh RK (2015) Mapping of Quantitative Trait Loci Associated with Reproductive-Stage Salt Tolerance in Rice. Journal of Agronomy and Crop Science, DOI 10.1007/s00122-013-2139-0.
- Ismail AM, Singh US, Singh S, Dar MH and Mackill DJ (2013) The contribution of Submergence-Tolerant (Sub1) rice varieties to food security in flood-prone rainfed lowland areas in Asia. Field Crops Research 152:83-93.
- Ismail AM, Thomson MJ, Singh RK, Gregorio GB and Mackill DJ (2008) Designing rice varieties adapted to coastal areas of South and Southeast Asia. Journal of Indian Society of Coastal Agricultural Research 26: 69–73.
- Kumar V, Singh A, Amitha Mithra SV, Krishnamurthy SL, Parida SK, Jain S, Tiwari KK, Kumar P, Rao AR, Sharma SK, Khurana JP, Singh NK, Mohapatra T (2015) Genome-wide association mapping of salinity tolerance in rice (*Oryza sativa*). DNA Research 2015: 1–13.
- Niroula RK, Pucciariello C, Ho VT, Novi G, Fukao T and Perata P (2012) *SUB1A*-dependent and -independent mechanisms are involved in the flooding tolerance of wild rice species. Plant Journal 72:282-293.
- NRRI Annual Report 2016-2017 (2017) ICAR-National Rice Research Institute, Cuttack, Odisha.
- Pandit A, Rai V, Bal S, Sinha S, Kumar V, Chauhan M, Gautam RK, Singh R, Sharma PC, Singh AK, Gaikwad K, Sharma TR, Mohapatra T and Singh NK (2010). Combining QTL mapping and transcriptome pro Wling of bulked RILs for identification of functional polymorphism for salt tolerance genes in rice (Oryza sativa L.). Molecular Genetics and Genomics 284:121–136, DOI 10.1007/s00438-010-0551-6.
- Platten JD, Egdane JA and Ismail AM (2013) Salinity tolerance, Na+ exclusion and allele mining of *HKT1;5* in *Oryza sativa* and *O. glaberrima*: Many sources, many genes, one mechanism?. BMC Plant Biology 13:32.
- Pradhan SK, Nayak DK, Mohanty S, Behera L, Barik SR, Pandit E, Lenka S and Anandan A (2015) Pyramiding of three bacterial blight resistance genes for broad-spectrum resistance in deep water rice variety, Jalmagna. Rice 8:19, DOI 10.1186/s12284-015-0051-8.
- Ren Z H, Gao J P, Li L G, Cai X L, Wei H, Chao D Y, Zhu M Z, Wang Z Y, Luan S and Lin H X (2005) A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nature Genetics 37:1141–1146.
- Saha S, Mahata KR, Sen P, Dani RC, Marndi BC and Singh DP (2008) Improving rice productivity in salt-affected coastal soils. CURE and CPW&F Project Bulletin No. 1 (in English and Oriya), Central Rice Research Institute, Cuttack.



- Septiningsih EM, Ignacio JCI, Sendon PMD, Sanchez DL, Ismail AM, Mackill DJ (2013) QTL mapping and confirmation for tolerance of anaerobic conditions during germination derived from the rice landrace. Theoretical and Applied Genetics 126:1357–1366
- Singh R, Singh Y, Xalaxo S, Verulkar S, Yadav N, Singh S, Singh N, Prasad KSN, Kondayya K, Rao PVR, et al. (2016) From QTL to variety-harnessing the benefits of QTLs for drought, flood and salt tolerance in mega rice varieties of India through a multi-institutional network. Plant Science 242:278-287, doi: 10.1016/j.plantsci.2015.08.008.
- Singh D P, Mahata K R, Sen, P, Saha S and Reddy J N (2009) Finale project Report on "Development of technologies to harness the productivity potential of salt-affected areas of the Indo-Gangetic, Mekong and Nile river basins. CGIAR Challenge Program on Water and Food Project Report series, www.waterandfood.org.
- Tian L, Tan L, Liu F, Cai H and Sun C (2011) Identification of quantitative trait loci associated with salt tolerance at seedling stage from *Oryza rufipogon*. Journal of Genetics and Genomics 38:593-601.
- Wassmann R, Jagadish SVK, Sumfleth K, Pathak H, Howell G, Ismail A, Serraj R, Redona E, Singh RK, Heuer S (2009) Regional vulnerability ofclimate change impacts on Asian rice production and scope for adaptation. Advances in Agronomy 102: 91–133.
- Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC and Mackill DJ (2006) Sub1A is an ethylene-response-factor- like gene that confers submergence tolerance to rice. Nature 442:705-708.\*



# Harnessing Heterosis in Rice for Enhancing Yield and Quality

## RL Verma, JL Katara, RP Sah, M Azharuddin TP, S Samantaray, S Sarkar, BC Patra, A Anandan, RK Sahu, AK Mukherjee, SD Mohapatra, S Roy, A Banerjee and ON Singh

## SUMMARY

Heterosis is a solitary means of exploiting hybrid vigour in crop plants. Given its vield advantage and economic importance several hybrids in rice have been commercialized in more than 40 countries, which creates a huge seed industry worldwide. India has made commendable progress and commercialized 101 three-lines indica hybrids for different ecology and duration (115-150 days) which accounted 6.8% of total rice area in the country. Besides, several indigenous CMS lines developed in diversified genetic and cytoplasmic background are being utilized in hybrid rice breeding. NRRI has been pioneering to start with the technology, has developed three popular rice hybrids viz. Ajay, Rajalaxmi and CR Dhan 701 for irrigated-shallow lowland ecosystem. Biotechnological intervention has supplemented immensely in excavating desirable genomic regions and their deployment for further genetic enhancement and sustainability in rice hybrids. Besides, hybrid seed production creates additional job opportunity (100-105 more-man days) and comparatively more net income (70% more than production cost) than HYVs. Hence, this technology has great scope for further enhancement in *per se* rice productivity and livelihood of the nation.

## **1. INTRODUCTION**

Heterosis is the superiority of F<sub>1</sub> offspring over either parent, a solitary means of exploiting hybrid vigour in crop plants. This phenomenon has benefited agriculture and fascinated geneticists for over 100 years for development of superior cultivar in many crops. Suitable allelic combination and manipulation has made yield advantage in hybrid than HYVs. It covers large acreage for many crops including rice and has fundamentally affected agricultural practices and the seed industry in the world. Heterosis had been exploited in various practical ways for centuries before Darwin provided an early scientific description of heterosis in maize. In rice, heterosis was first reported by Jonse (1926 AD). However, owing to its self-pollinating nature (0.3 -3.0% out-crossing), heterosis could be realized during middle of second half of the twentieth century after identification and development of CMS source. Subsequently, the China, under the leadership of Yuan Long Ping, started work on the development of hybrid rice with a vision to make it possible to be commercial. He identified a natural male sterile mutant plant in *indica* rice and pollen abortive genotypes in the natural population of wild rice (Oryza rufipogon; 1970 Li), which later served as donor of male sterile source (male sterile cytoplasm) for CMS development. In 1973,



through recurrent back-cross breeding several promising *indica* wild abortive CMS viz. Erjiunan1A, Zhenshan 97A and V20A CMS-WA and good restorers viz., Taiyin1, IR4 and IR1 were developed. Later during 1974, first *indica* rice hybrid Nanyou 2 was released for cultivation in China. Afterward, relatively more heterotic hybrid rice breeding approaches like two-line system (1987 AD) and super hybrids (1996 AD) were adopted which supplemented substantially toward Chinese food security and livelihood.

In India, systematic research on hybrid rice was initiated in 1989 when Indian Council of Agricultural Research (ICAR) launched a special goal oriented and time bound project 'Promotion of Research and Development Efforts on Hybrids in Selected Crops' for rice at 12 network centres. After four years of meticulous research (1989-93), first hybrid rice was released in Andhra Pradesh in 1993-94 and India became the second country after China to develop and commercialized hybrid rice. So far, 101 rice hybrids (33 from public organization and 68 from private sector) were developed, suitable for different ecology and duration ranging from 115-150 days, covering 3.0 mha which accounted 6.8% of total rice area in India.

Hybrid rice technology is impressive as it enhances farm productivity of 15-25% more than HYVs. Given its yield advantage and economic importance, several hybrids in rice have been commercialized in more than 40 countries, which creates a huge seed industry world-wide. Moreover, this venture also has great service opportunity, creates additional employment for 100-105-man days/hectare in seed production. However, it has some limitations in generation of hybrids, seed production and marginal heterosis. Success of hybrid depends on their parental combination, adaptability and allelic interactions, hence, faces several problems like unstable male sterility, non-abundancy in cytoplasmic diversity, inherited CMS load, low seed producibility in seed parent, poor grain and eating quality, lack of responsive parents for biotic and abiotic stresses,

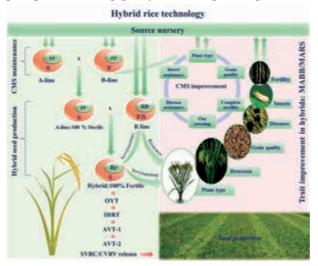


Fig. 1. A schematic representation of hybrid rice technology (seed production, trait improvement, yield evaluation etc.).

hybrid sterility, marginal heterosis in indica hybrids etc. This chapter deals with information on (i) status of hybrid rice research (ii) breeding system and methods involved in hybrid rice development and production (iii) trait specific parental line improvement (iv) molecular dissection of genes and QTLs for parental line improvement and (v) economic opportunity (Fig. 1).



## 2. BREEDING COMPONENT AND SYSTEM IN HYBRID RICE DEVELOPMENT

Rice is a strict self-pollinated crop; commercial exploitation of heterosis requires some parental specificity which could excludes manual emasculation. The invention of naturally occurred male sterility (MS) in rice thus played substantial role in realization of heterosis in rice. Following are the genetic tools as mentioned in various heads are required for development and commercialization of hybrid in rice:

### 2.1. Male sterile system

The male sterility (MS) in plants is the condition where male reproductive organ, anthers lose their ability to dehisce and produce viable pollen and thus encourage allo-gamous nature of reproduction. This is crucial breeding tools to harness heterosis that exclude additional efforts of emasculation which is cumbersome process. In plants male sterility is conditioned either by mitochondrial or nucleus genome or in associations. The male sterility in plant was first observed by Joseph Gottlieb Kolreuter in 1763 and later it was reported in > 610 plant species. In rice, it was reported by Sampath and Mohanthy (1954) at ICAR-NRRI (formerly CRRI), Cuttack by studying the differences in male fertility in reciprocal crosses of *indica/japonica* rice lines. The male sterility in plant is found to be determined by several biological as well as environmental factors. In rice, it is conditioned either by cytoplasmic genes in association with nuclear genes (CMS) or nuclear genes alone (GMS) which cause abnormal development in sporogenous tissue (either sporophytic or gametophytic tissue). The sporophytic male sterility is governed by genetic constitutions of sporogenous tissues like tapetal and meiocytes which creates improper nourishing to developing microspores and cause pollen abortion, whereas in gametophytic male sterility, microspore and pollen development get affected. Sporophytic male sterility is quite useful in hybrid rice breeding as it gets fertile in heterozygous state and encourages complete fertility in resulting hybrids. To date several types of male sterile system viz. cytoplasmic male sterile (CMS), environment sensitive male sterile (GMS) viz. thermo-sensitive genetic male sterility (TGMS), photo-sensitive genetic male sterility (PGMS) and reverse photo-sensitive genetic male sterility (rPGMS) etc. have been identified and substantially being utilized in hybrid development (Table 1).

### 2.2. Diversity in male sterile system and their mechanism

Cytoplasmic male sterility is a maternally inherited trait caused by improper communication between cytoplasmic and nuclear genome (Chen 2014). Gene(s)/genic block (s) conditioned cytoplasmic male sterility are chimeric construct, evolved due to rearrangement of the mitochondrial genome (Fig. 2). In rice, several types of cytoplasmic male sterility have been identified and characterized, having diversified mechanism in MS expression. Wild abortive (WA-CMS), a sporophytic MS system widely utilized in hybrid development. It is found to be caused by a constitutive mitochondrial gene WA352c located down stream of *rpl5* (comprised four mitochondrial genomic segments, *orf284, orf224, orf288* and *cs4-cs6*) and encodes a

NRRI

Contd....

lable 1. Cytoplasi	lable 1. Cytoplasmic diversity in rice CMS.	MS.		
CMS group	Associated ORF	Protein	Cytoplasm source	Representative CMS-line
1. Cytoplasmic male sterile line	sterile line			
a. BT-CMS and their lineage	- lineage			
BT-CMS (G)	B-atp6-orf79	Membrane protein	Chinsurah Boro II/Taichong 65	Liming A, Xu 9201A
LD-CMS (G)	UK	UK	Lead Rice (Burmese <i>indica</i> variety) x Fujisaka 5 ( <i>japonica</i> variety)	Fujisaka 5A
Dian1-CMS (G)	UK	UK	Yunnan high altitude landrace rice (indica) cytoplasm	Yongjing2A, Ning67A
HL-CMS (G)	atp6-orfH79	Membrane protein	Red-awned wild rice (Oryza rufipogon) cytoplasm	Yuetai A, Luohong 3A4
b. WA-CMS and their lineage	ir lineage			
WA-CMS (S)	rp15-WA352	Membrane protein	Wild abortive rice (Oryza ruftpogon) cytoplasm	Zhenshan97 A, V20AIR58025A, CRMS31A etc.
Kalinga-I-CMS (S)	UK	UK	Kalinga-I (indica) cytoplasm	CRMS 32A
D-CMS (S)	UK	UK	Indica rice Dissi D52/37	D-Shan A, D62A
DA-CMS (S)	UK	UK	Dwarf abortive rice (Oryza rufipogon) cytoplasm	Xieqingzao A
GA-CMS (S)	UK	UK	Gambiaca (indica) cytoplasm	Gang 46A
ID-CMS (S)	UK	UK	Indonesia paddy rice (indica) cytoplasm	II 32A, You1A
K-CMS (S)	UK	UK	K52(japonica) cytoplasm	K-17A
CMS-RT102 (S)	rpl5-orf352	Membrane protein	Oryza rufipogon, W1125	RT102A
CMS-RT98A (G)	orf113-atp4-cox3	Membrane protein	Oryza rufipogon Griff, W1109	RT98A
LX-CMS	UK	UK	Luihui rice (indica) cytoplasm	Yue 4A
Maxie-CMS	UK	UK	MS mutant of Maweizhan ( <i>indica</i> ) with Xieqingzao ( <i>indica</i> )	Maxie A
NX-CMS	UK	UK	Selected from F2 male sterile plants in the progeny of Wanhui 88 ( <i>indica</i> ) x Neihui 92–4 ( <i>indica</i> ) nucleus	Neixiang 2A, Neixiang 5A

Table 1. Cytoplasmic diversity in rice CMS.

	HIE	
S-line		

CMS group	Associated ORF	Protein	Cytoplasm source	Representative CMS-line
Y-CMS	UK	UK	Yegong (indica landrace) cytoplasm	Y Huanong A
CW-CMS (G)	orf307	Mitochondrial protein	Oryza rufipogon Griff.	IR24A, IR64A
2. Environment sens	. Environment sensitive genetic male sterility (EGMS)	ility (EGMS)		
PGMS	pms3	Noncoding RNA	Nongken 58S, PGMS mutant of <i>japonica</i> cultivar Nongken 58	7001S, N5088S
P/TGMS	p/tms12-1	noncoding RNA	Photoperiod and temperature sensitive genic male	Pei'ai 64S
TGMS	<i>tms5</i> , <i>RNase</i> Z <sup>S1</sup> (loss in function)	Nuclease enzyme	sterile (P/TGMS) derived from Nongken 58S Spontaneous TGMS mutants of Annong S-1 and Zhu 1S	Guangzhan 63S5, Xinan S
rPGMS	csa OsMST8	MYB transcript regulator	Carbon starved anther (csa) mutant of japonica cultivar 9522	9522S
Note: 'S' stand for s	porophytic male steril	ity and 'G' stand to gai	Note: 'S' stand for sporophytic male sterility and 'G' stand to gametophytic male sterility.	



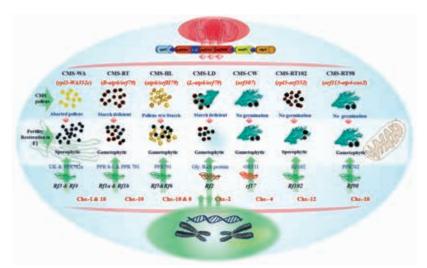


Fig. 2. Schematic presentation of rice CMS types. Where, WA stand for wild abortive, BT is for boro type, HL for Honglian, LD for lead rice, CW for Chinese wild rice, RT102A and RT98A, respectively.

352-residue putative protein with three transmembrane segments. The WA352c inhibits nuclear-encoded mitochondrial protein COX11 (essential for the assembly of cytochrome c oxidase, TCA) and triggered premature tapetal programmed cell death and pollen abortion (Tang et al. 2017). In contrast, BT-CMS is a gametophytic MS reported in the Indian rice variety, Chinsurah Boro-II in which pollen development get arrested at the tri-nucleate stage. The mitochondrial chimeric (dicistronic) gene B*atp6-orf79* encodes a transmembrane protein, cytotoxic peptide *ORF79* (Wang 2006) which accumulates preferentially in the microspore, was found to be responsible for male sterility. The orf79 reside downstream to the atp6 and interact with P61 and mitochondrial complex III and impair the activity of this complex which lead to dysfunctional energy metabolism and elevate oxidative stress and thus causing sterility. However, in HL-CMS, which is also a gametophytic MS system, pollen development gets arrested at di-nucleate stage. A chimeric aberrant transcript of the mitochondrial gene atp6-orfH79 located downstream of atp6 is confirmed as candidate gene of this MS. Transcript of orfH79 gene preferentially accumulates in mitochondria which impairs mitochondrial function through its interaction with P61, a subunit of electron transport chain (ETC) complex III (Wang et al. 2013) and leads male sterility. Male sterility in CW-CMS is conditioned by mitochondrial orf307 which expressed preferentially in anther tissue, indicating the presence of anther-specific mitochondrial retrograde regulation of nuclear gene expression. It is a gametophytic MS in which pollen grain appears normal but lacks the ability to germinate.

### 2.3. Genetic male sterility

Genetic male sterility (GMS) in rice is conditioned by recessive nuclear genes and showing normal Mendelian inheritance. Owing to difficulties in their maintenance (occurrence of only 50% sterility in  $F_1$ ), GMS could not be part of rice hybrid breeding



programme. Some GMS lines has shown threshold nature in MS expression where male sterility occurs in specific environmental regime (high temperature and long day length); hence called environment sensitive genetic male sterile (EGMS). The GMS line shows male sterility at elevated temperature i.e. >30 °C is called temperature sensitive male sterility (TGMS) whereas male sterility in long day length i.e. >13.5 hrs is called photoperiod-sensitive genetic male sterility (PGMS). The male sterility in EGMS line is found to be revert into male fertile in favourable temperature (<30 °C) and day length (<12.5 hrs) which provide its unique opportunity to be utilized in hybrid rice breeding programme. Some lines, such as Pei'ai 64S, are male sterile under both long day and high temperature conditions and are referred to as P/TGMS lines. The majority (>95%) of the EGMS lines utilized in hybrid rice production were derived from three independent progenitors i.e. PGMS line Nongken 58S (NK58S), TGMS lines Annong S-1 and Zhu1S. Many lines derived from NK58S were P/TGMS or even TGMS (e.g., Guangzhan 63S), but the underlying mechanism leading to such dramatic changes has yet to be revealed. Recently, a novel type of EGMS (csa-carban starved anther mutant) in rice called rPGMS (reverse PGMS), shows normal male fertility under long day conditions (>13.5 hrs) and male sterile under short day conditions (<12.5 hrs) is identified. This is found to be suitable for seed production of two-line hybrids in tropics and subtropics (Zhang et al. 2013).

## 2.4. Transgenic cytoplasmic male sterility

The genetically engineered male sterile line M2BS in rice is developed by transformation of *indica* rice maintainer M2B with partial-length HcPDIL5-2a (Hibiscus cannabinus protein disulfide isomerase-like) genetic construct. Male fertility in this CMS is reported to be arrested due to tapetum degeneration which leads pollen abortion. Hereditary analysis indicated that the male sterility of M2BS was a maternally inherited inability could be affirmed as a type of cytoplasmic male sterile (CMS). Besides, by combining cysteine-protease gene (BnCysP1) of *Brassica napus* with rice anther-specific P12 promoter (promoter region of *Os12bglu38* gene), a transgenic MS system was successfully created which is restored by transgenic rice plants carrying BnCysP1Si silencing system (Rao et al. 2018). Zhou and co-workers (2016) developed 11 "transgene clean" TGMS lines by editing most widely utilized TGMS gene *tms5* through CRISPR/Cas9.

### 2.5. Genetics of fertility restorer gene

Cytoplasmic male sterility in rice is found to be restored by nuclear genome i.e. mono or oligo nuclear loci called restorer gene. In rice a total of ten *Rf* genes (*Rf1a*, *Rf1b*, *Rf2*, *Rf3*, *Rf4*, *Rf5*, *Rf6* and *Rf17*, *Rf98* and *Rf102*) have been identified, of those seven (*Rf1a*, *Rf1b*, *Rf2*, *Rf4*, *Rf5*, *rf17* and *Rf98*) are characterized. All Rf genes are found to be dominant in nature (except *rf17*, restore fertility in CW-CMS) which can restore male fertility in heterozygous state. Restorer genes are very specific to male sterile genome in the mechanism of fertility restoration. Genes *Rf1a* and *Rf1b* (Chr.-10) encodes pentatricopeptide-repeat (PPR) containing proteins, have functional affinity of fertility restoration in *BT-CMS*; RF1A promotes endo-nucleolytic cleavage of the



atp6-orf79 mRNA and RF1B promotes degradation of atp6-orf79 mRNA (Wang et al. 2006) and revert the male sterility into fertility. Whereas, HL-CMS is restored either by Rf5 or Rf6 gene, these genes can produce 50% normal pollen grains in F1 plants individually, however both genes in complementation could restore more than 80% spikelets fertility in hybrids. The Rf5 encodes a PPR family protein PPR791 which makes a restoration of fertility complex (RFC) with glycine rich protein GRP162 and bind to the atp6-orfH79 transcripts. The RFC cleave the aberrant transcript of atp6orfH79 at 1169 nucleotides position (Hu et al. 2012). The Rf6 gene encodes a novel PPR family protein (duplicate PPR motif 3-5) which in association with hexokinase (osHXK6) targets mitochondria and process defective transcript of atp6-orfH79 at 1238 nucleotide position. Thus, PPR protein family cause editing of aberrant transcript, inhibit their translation and at the end fertility restoration (Huang et al. 2015). Besides, male fertility in WA-CMS is found to be counteracted by Rf3 and Rf4 genes (chrom. -1 and 10, respectively). The gene Rf3 and Rf4 encodes a pentatricopeptide protein (PPR) where RF4 cleave the abnormal WA352 transcript and RF3 supress translation of WA352 into polypeptide and helps in restoring fertility in WA-CMS. Fertility in LD-CMS is reported to be restored by either Rf1 or Rf2. The Rf2 gene encodes a mitochondrial glycine-rich protein; replacement of isoleucine by threonine at amino acid 78 of the RF2 protein causes functional loss of the rf2 allele. Moreover, CW-CMS is restored by a single recessive nuclear gene, rf17 which is a retrograderegulated male sterility (rms) gene (Toriyama et al. 2016) (Table 2).

## 2.6. System of hybrid rice breeding

Commercial hybrid seed production in rice where natural out-crossing (ranged only 0.3-3.0 %) is very low, is a cumbersome and expansive task. To be practical and readily adoptable, it requires some specific parental requirements and agro-management practices. Invention of male sterile lines thus provided unique opportunity to start with the technology in rice. Based on mechanism of male sterility, threshold nature in male sterility expression and number of parental lines used, three type of hybrid seed production system namely three- line system (involving three parents, A, B and R), two-line system (two parents, A and R) and one-line system (apomictic based) exist. Among them, CGMS based three lines system is more suitable, hence widely utilized (>90% of world's hybrids developed utilizing this) in hybrid rice varietal development and seed production.

**2.6.1. Three-line system (involving three parents, A, B and R):** Three-line hybrid system involves three parents such as male sterile line (A-line, cytoplasmic Male sterile), B-line (maintainer) and R (restorer) lines and two steps in seed production i.e. CMS multiplication and hybrid seed production under strict isolation (spatial or temporal or physical barrier). Male sterile line (A-line), because of their eliminated manual emasculation needs, served as seed parent and facilitates large scale seed production. A suitable CMS line to be utilized as seed parent should have complete and stable male sterility, substantial seed producibility, wide compatibility and good combining ability with minimum CMS load. The wealthy panicle and narrow semi erect leaf configuration in seed parent has additional impact, assure more seed

HIE SHALL	
-----------	--

Table	table 2. Nestorer genes III rice plants.							
S. No.	S. No. Rfgenes	Locality	Marker	CMS system	Restorer line	Causative gene	Encoded product Reference	Reference
	Rf1a, Rf1b	Chr-10	InDel-Rf1a	CMS-BT	BTR, IR24, MTC10R;	PPR8-1, PPR791, <i>Rf1A, Rf1B</i> C 9083	PPR	Tao et al. 2013
7	Rf2	Chr-2	CAPS42-1	CMS-LD	Kasalath, Minghui 63	LOC_Os02g 17380.1	Gly. Rich protein Itabashi et al. 2011	Itabashi et al. 2011
ω	Rf3	Chr-1	DRRM-Rf3-10	CMS-WA	Swarna, PUSA 33,	1	PPR	Katara et al. 2017
4	Rf4	Chr-10	RM6100	CMS-WA	IR 24, Pusa 33, PPR782a CRL 22R	PPR782a	PPR	Katara et al. 2017
5	Rf5(t)	Chr-10	RM3150	CMS-HL	Milyang 23	PPR791	PPR	Liu, 2004
9	Rf6	Chr-10 & 8	RM5373	CMS-HL	I	ı	ı	Liu, 2004
2	rf17	Chr-4	AT10.5-1, SNP 7-16	CMS-CW	CWR	PPR2	RNA interference- Fujii et al. 2005	Fujii et al. 2005
∞	Rf98	Chr10	UK	CMS-RT98A	RT98C	PPR762	PPR	Igarashi et al. 2016
6	Rf102	Chr12	UN	CMS-RT102A	RT102C, K102-Oryza rufipogon. T	UK	UK	Okazaki, 2013

Harnessing Heterosis in Rice for Enhancing Yield and Quality



production. In Indian perspective where, hybrid seed production is major dilemma, generally keen to *Rabi* season, hence, CMS lines should have substantial cold tolerance at seedling stage and heat at flowering stage.

The maintainer (B-line) on the other hand is an isogenic to the CMS line (differs only for fertility/sterility) in their genetic constitution, able to produce functional pollen and maintain the sterility in male sterile line/seed parent. The maintainer line can maintain 100% male sterility in seed parent thus utilized to perpetuate CMS with their inherent male sterile ability.

In contrast, restorer line can restore male fertility in F<sub>1</sub>s produced on male sterile parent, thus utilized as pollen parent in hybrid seed production. A good restorer should have substantial genetic distance with seed parent which is prerequisite and major determinant of the extent of heterosis in hybrids (more genetic distance more heterosis and *vice -versa*). Restorer is the major contributor of heterosis in three-line hybrids, hence, should have good combining, strong fertility restoration ability (dominant *Rf* gene(s) responsible for fertility restoration in CMS). Besides, restorer line with ideal in plant type, acceptable grain quality parameters, substantial source-sink balance, heavy pollen load and broad spectrum of resistance/tolerance against multiple biotic/abiotic stresses is imperative in maximization of genetic gain in hybrids.

**2.6.2. Two-line system (involve two parent A and R):** Two-line system is a simple and more efficient hybrid breeding system in rice, involves only two parents i.e. A and R line in seed production. This is a threshold of genetic male sterility (EGMS) based hybrid rice breeding system where male sterility is conditioned in specific environmental regimes such as long photoperiod (>13.5 hrs day length) and at elevated temperature (>30 °C). In this system male sterile parents are to be maintained by selfing under favourable conditions (below critical sterility point i.e. <30 °C temperature and at below CSP of photoperiod length, <12.5 hrs.).

Two-line hybrid seed production system is easy and effective alternative to CMS, has specific advantages as requires only one step for seed production. In this system any good combiner genotype irrespective of their fertility restoration ability can be utilized as pollen parent. EGMS system is normal, does not exert any ill effect on the growth and development of carrier plant, thus exploit comparatively higher extent of heterosis (up to 5-10%) in  $F_1$  than the CGMS based three-line hybrids. The EGMS traits are governed by major genes, thus easily transferable to any genetic background which helps in reducing potential genetic vulnerability among the hybrids. Because of its eliminating needs for restorer genes in the male parents, this is ideal for developing inter-subspecific (*indica/ japonica*) hybrids.

**2.6.3. One-line system (apomictic based):** In this system, seeds of rice hybrid once generated need not to be further produced in hybrid seed production plot. This system is solely based on apomixes phenomenon (embryo developed apart from mixing of sexual gametes/fertilization) where embryo developed without fertilization. In this system hybrid seeds once generated will be maintained through apomixes in their original heterozygous form. This system is yet to be explored in rice, in future it is anticipated to be realized in commercial use.



# 3. PROGRESS IN HYBRID RICE RESEARCH AND DEVELOPMENT

#### 3.1. International status

Hybrid technology is one of the greatest innovations in the modern era, contributed greatly in yield enhancement in several important crops. Over the decades of rigorous research Chinese could develop parental lines i.e. cytoplasmic male-sterile line, maintainer line and restorer line which assisted in the realization of heterosis exploitation in rice. Subsequently, hybrid seed production system was refined and world's first hybrid rice was released for commercial cultivation during 1974 AD. The first generation wild abortive CMS line i.e. Zhenshan 97A was widely utilized and several elite hybrid rice varieties were commercialized. Besides, several CMS with altered genetic mechanism of male sterility expression were also identified and characterized.

At beginning, low seed producibility with WA-CMS was a concern for its commercialization. However, with the keen interest of agronomist, management practices for hybrid seed production were sustainably rationalized. The Chinese government has supported this venture in pilot mode and established large and effective hybrid rice seed businesses in the late 1970s at all levels. Besides, intensive mechanization of hybrid seed production helped in modification of planting ratio (2R: A as 6–8 rows to 40–80 rows) and reducing the cost of production. Therefore, China could achieve seed yield by 2.7–3.0 t/ha on large scale in hybrid rice seed production which is further enhanced to 3400 Kg/ha and maximize their acreage.

Over past three decades hybrid rice varieties have been substantial for national food security in the China which accounted for approximately 57% of the total 30million-hectare rice planting area. The Ministry of Agriculture, China has launched project on super hybrid rice development during 1996 which resulted altogether 73 super hybrids (52 three-line hybrids and 21 two-line hybrids) for commercial cultivation. Super hybrid P64S/E32 released recently has recorded new height of yield potential of 17.1 t/ha with some striking characteristics (Yuan et al. 2017).

Beside China, this technology has also been introduced and promoted by more than 40 countries around the world. At beginning, IRRI helped technically and supplied prerequisite parental materials. Later, most of the countries could establish their own hybrid rice breeding programme and developed several heterotic hybrids. India was the second country after china that adopted this technology in 1989 and made substantial progress. At present, hybrid rice covers around 3.0 mha in India that has 6.8% of total rice area. Vietnam was the next to adopt this technology in 1992, harnessing yield of 6.3–6.8 t/ha from 0.7 mha, which covers around 10% of their rice area. In Philippines it was introduced in 1993. Several popular hybrids like Magat, Mestizo, Mestizo 2, Mestizo 3, Bigante, Magilla, SL8H, Rizalina 28 etc. were developed and commercialized. Hybrid seed production in Philippines has been handled by 'seed growers' cooperatives, who is to produce around 60-70% of them. In Bangladesh several rice hybrids were introduced and commercialized from China, India and Philippines. They are almost self-sufficient in hybrid seed production, producing



around 8000 tons to cover about 800,000 ha. In order, Indonesia also has substantial hybrid rice area, developed several good rice hybrids like Hipa7, Hipa 8, Hipa9, Hipa10, Hipa11, Hipa12 SBU, Hipa13, Hipa14 SBU, Hipa Jatim1, Hipa Jatim2 and Hipa Jatim3 were extensively commercialized, having yield superiority of 0.7-1.5 tons/ha over the lowland inbred varieties.

United States of America has adopted this technology during 2000, has developed and commercialized several two-line and three-line hybrids. Most of the hybrid rice cultivars in USA employed Clearfield (CL) technology offering selective control of weedy red rice. Rice hybrids viz. Clearfield XL729, Clearfield XL745, Clearfield XP756 (a late maturing) and Clearfield XP4534 (new plant type) has shown yield advantage ranging from 16-39% over inbred cultivars are being commercialized by RiceTec.

### 3.2. National status

In India, systematic hybrid rice research was initiated in 1989. The first hybrid rice was released in Andhra Pradesh during 1993-94 and India became the second country after China to commercialize hybrid rice. India has made substantial progress and developed total 101 (*indica/indica*) rice hybrids having 15-20% yield superiority with 115-150 days duration for various rice ecosystems. Recently, Savannah Private Limited from India has made another landmark by developing 2 two-line rice hybrids viz. SAVA-124 and SAVA-134 for commercial cultivation. In addition, more than 100 CMS in diversified genetic and cytoplasmic background have been developed and utilized. Amongst, the promising CMS lines CRMS 31A, CRMS 32A, CRMS 8A, PMS10A, PMS 17A, APMS 6A, DR8A, PUSA 5A, PUSA6A, RTN 12A etc. are substantially being utilized in development of rice hybrids in India and abroad. Notably, medium duration seedling stage cold tolerant CMS, CRMS 32A, developed at NRRI under Kalinga-I cytoplasm is more suitable for development of hybrids for *boro* ecosystem. Two popular hybrid rice varieties namely Rajalaxmi and KRH 4 were developed using CRMS 32A as one among the parent.

Initially, this programme was technically supported from the International Rice Research Institute (IRRI), Philippines and Food and Agriculture Organization (FAO), Rome; and financially supported from United Nations Development Programme (UNDP), Mahyco Research Foundation, World Bank funded National Agricultural Technology Project (NATP) and IRRI/ADB projects on hybrid rice. Scaling up and popularization of hybrid rice in India was further taken over by union government through various national schemes like RKVY, BGREI etc. To further invigorate the hybrid rice research and development, ICAR has launched a 5-year consortium research platform project 'ICAR - Consortium Research Platform on Hybrid Crops Hybrid Technology for Higher Productivity in Selected Field and Horticultural Crops, at nine research centres. Indo-ASEAN group has also started funding for genetic diversification of parental lines and development of inter-subspecific hybrids in India and member's countries.

Hybrids released in India having unambiguous specificity like specific to ecosystem, tolerant to several abiotic/ biotic stresses and consumer preferences (Table 3). These hybrid varieties can be utilized to up scale the hybrid rice cultivation and productivity enhancement *per se* in the respective area.



S. No.	Stress	Promising hybrids
1	Rain-fed upland	DRRH-2, Pant Sankar Dhan-1, Pant Sankar Dhan-3, KJTRH-4
2	Salinity	DRRH-28, Pant Sankar Dhan-3, KRH-2, HRI-148, JRH-8, PHB-71, Rajalaxmi
3	Alkalinity	Suruchi, PHB-71, JKRH-2000, CRHR-5, DRRH-2, DRRH-44, Rajalaxmi
4	Boro/Summer season	Rajalaxmi, CRHR-4, CRHR-32, NPH 924-1, PA 6444, Sahyadri, KRH 2
5	BB resistant	BS 6444G, Arize Prima, Rajalaxmi, Ajay, CR Dhan 701, PRH 10 etc.

### Table 3. Rice hybrids tolerant to various stresses

Hybrids like CRHR 105, CRHR 106, 25P25, 27P31 are suitable for high temperature regime which has more deleterious effect on seed development in hybrids. The hybrid varieties, US 382, Indam 200-17, US 312, DRRH3, JKRH 401 having high N use efficiency thus found suitable for cultivation in N deficient soil. Besides, hybrids PNPH 24, RH 1531, Arize Tej are under mid-early maturity group which can sustain substantially under drought situations. The problems of coastal and shallow lowland ecosystem sharing around 32% of total rice area can be addressed by adopting long duration hybrids like CRHR 32, Arize Dhani, CRHR 34, CRHR 102 and Sahyadri 5 (Table 4).

### Table 4. Hybrids suitable for specific condition

Aerobic condition	PSD 3, PSD 1, Rajalaxmi, Ajay, ADTRH 1, PRH 122, DRRH 44, HRI 126, JKRH 3333, KRH 2,
Early duration	CRHR 105, CRHR 106, 25P25, 27P31 (heat tolerant), US 382, Indam 200-17, US 312 <sup>+</sup> , DRRH3, JKRH 401high N use efficient; PNPH 24 & RH 1531, Arize Tej-mid early-drought tolerant; DRRH2, KJTRH-4 (upland)
Long duration	CRHR 32, CRHR 34, CRHR 100, Sahyadri 5,
SRI	TNRH CO-4, KRH 4
Idly making	VNR 2355+
MS grains	CRHR 32, DRRH 3, 27P63, 25P25, Suruchi
Aromatic	PRH 122 (mild aroma), PRH 10

### 3.3. ICAR-National Rice Research Institute's contribution

The ICAR-National Rice Research Institute, Cuttack has been pioneer to start with the technology in late of 7<sup>th</sup> decade of last century, quite before the beginning of their project mode programme in 1989 by ICAR. In the beginning, ICAR-NRRI has acquired all the prerequisite materials (CMS lines viz. V 20A, Yar Ai Zhao A, Wu10A, MS 577A, *Pankhari* 203A, V 41A, Er-Jiu nan A, respective maintainers, 9 other maintainers and thirteen restorers) from the IRRI (NRRI annual report 1981-82). Systematic hybrid rice breeding was initiated in interdisciplinary mode with objectives to develop desirable parental lines viz., cytoplasmic genetic male sterile (CGMS)

lines, maintainers and restorers for development of rice hybrids for irrigated and shallow submergence. The farmers in the rain fed shallow lowland ecosystem would be extremely benefited if the hybrid rice technology can be extended to this ecosystem, which need hybrids of Swarna duration. Keeping in views, ICAR-NRRI has developed three rice hybrids viz. Ajay, Rajalaxmi and CR Dhan 701 for this fragile ecosystem. Among them CR Dhan 701 is the country's first long duration hybrid, substitute for popular variety Swarna. Besides, NRRI has developed several promising CMS lines which have stable male sterility (WA, Kalinga-I and O. perennis etc. cytoplasmic background), maintainers and effective restorers. More than 45 CMS lines in diverse genetic and cytoplasmic background have been developed amongst, Sarasa A, Pusa 33A (WA), Annada A (WA), Kiran A (WA), Deepa A (WA), Manipuri A (WA), Moti A (WA), Krishna A (O. perennis), Krishna A (Kalinga I), Mirai (Kalinga I), Padmini A, PS 92A and Sahabhagidhan A etc. are more prominent to be utilized in hybrid development. The medium duration CMS, CRMS 31A (WA) and CRMS 32A (Kalinga-I) are significantly utilized for hybrid development at NRRI and elsewhere in the country. The CRMS 24A and CRMS 40A, developed under the nucleus background of Moti and Padmini are found suitable for late duration hybrid breeding. Moreover, short duration CMS, CRMS 8A, CRMS 51A and CRMS 52A and CRMS 53A having drought tolerance are also being used for development of hybrids for drought prone ecosystem.

The latest release CR Dhan 701 (CRHR32) found suitable for irrigated-shallow lowland of Bihar, Gujarat and Odisha having MS grain type with an average yield capacity of 7.5 t/ha. This hybrid shows substantial tolerant to low light intensity, thus having great scope in eastern Indian states where low light limits the potential expression of hybrids/varieties during wet season. Moreover, hybrid Rajalaxmi (125-130 days) was developed utilizing native CMS line CRMS 32A, released by SVRC 2006/CVRC 2010 for irrigated-shallow lowland of Odisha and *boro* ecosystem of Odisha and Assam as it has seedling stage cold tolerance. Ajay is a medium duration with long slender grain type hybrid, released for cultivation in irrigated-shallow lowland of Odisha. As these hybrids are adaptable for eastern Indian climatic condition with assured remuneration, 12 private seed agencies over five states have commercialized them.

To make this technology more sustainable and amenable to farmers, trait development strategy among the parental lines becomes mandatory. The parents of ICAR-NRRI bred hybrids Ajay, Rajalaxmi and CR Dhan 701 has been improved for bacterial blight, the most devastating disease of rice (Das et al. 2016). The submergence and salinity are the major abiotic stresses occur frequently in rain-fed shallow lowland area and causes substantial yield loss in rice. Hence, to cope up with the problems, and make hybrid rice more sustainable during these adversity, ICAR-NRRI has successfully stacked submergence and salinity tolerant QTLs in the seed parents CRMS 31A and CRMS 32A. To enhance the seed producibility in seed parents, introgression of stigma exsertion trait from *O. longistaminata* into CRMS 31A and CRMS 32A, are under progress. To excavate the genetic region responding heterosis in rice, transcriptomic analysis of hybrids Rajalaxmi and Ajay are completed and interpreted. Availability of restorers for WA-CMS lines is very stumpy in nature, only



15% of total rice genotypes having the ability to restore complete fertility in WA-CMS based hybrid rice (Katara et al. 2017). Hence, good combiner genotypes having partial fertility restorers Mahalaxmi and Gayatri were improved by introgressing fertility restorer gene(s) *Rf3 & Rf4* through MABB approach. Further, to make clear cut identity and ensure pure seed of parents/hybrids to the stack-holder, 12 signature markers that unambiguously distinguish 32 rice hybrids were developed, which can be utilized for DNA fingerprinting and genetic purity testing of hybrids.

# 4. POTENTIAL APPLICATION OF OMICS APPROACHES IN HYBRID RICE BREEDING

Recent advancement in molecular biology has offered tremendous opportunities to the breeder and breeding *per se* in enhancement in their efficacy and speed up the varietal development process. It has diverse application like mapping, tagging, amplification-based cloning, gene pyramiding, marker assisted selection (MAS/MARS), fingerprinting applications including varietal identification, ensuring seed purity, phylogeny and evolution studies, diversity analysis and elimination of germplasm duplication. The progress in research related to application of DNA marker technology in hybrid rice improvement may be valuable in following way.

## 4.1. DNA fingerprinting and genetic purity testing

Varietal identity of hybrids and parents is imperative to assure the ownership (IPR issue) and pure seeds to the stakeholders. The genetic purity testing of hybrid seed is done by conducting Grow-Out-Test (GOT) which is time taking (needs one full growing season), tedious and very expensive. Molecular markers in this context found to be a suitable alternative, provide an unbiased means of identifying crop varieties. Amongst, available DNA-based markers, sequence tagged microsatellite (STMS) which are co-dominant in nature, are widely used for speedy genetic purity assessment of the hybrids and parental lines (Behera et al. 2012; Verma et al. 2017). Besides, ICAR-NRRI has developed another set of nine signature markers which can distinguish parents CRMS 31A, CRMS 32A; and hybrids Ajay, Rajalaxmi and CR Dhan 701, unambiguously.

### 4.2. Trait improvement in parental lines and hybrids

Hybrid rice has been one of the innovation that led the quantum jump in rice productivity last century. However, the challenge of meeting the increasing demand for rice and making hybrid more sustainable under impeding climatic changes, trait development in parental lines for ideal plant type with substantial yield, grain quality, and resistance/tolerance to multiple biotic and abiotic stresses is necessary. In this context, conventional breeding is more cumbersome, time taking and less précised. The advancement in molecular breeding techniques makes it convenient to improve the parents and hybrids for desirable traits with great precision. Marker assisted selection/MABB has provided strong utensils for indirect selection/trace the trait of interest at any plant growth stage. The bacterial blight and blast are the two-major

destructive diseases affecting rice plant at different growth stage and caused substantial yield loss. Resistant genes for BB diseases has been deployed successfully in popular hybrids Rajalaxmi, Ajay (Das et al. 2016), BS 6444G, PRH 10, (Basavaraj et al. 2009), Shanyou 63, Guangzhan 63-4S (Zhang et al. 2006, Huang et al. 2012); seed parent of CR Dhan 701, restorers Minghui 63 and Mianhui 725 (Das et al. 2016, Chen et al. 2014), Zhonghui 8006 and Zhonghui 218 (Cao et al. 2005) etc. The popular CMS line Rongfeng A (Fu et al. 2012), Pusa 6A female parent of popular basmati hybrid PRH 10 (Singh et al. 2015), RGD-7S and RGD-8S (Liu et al. 2008) were successfully stacked with blast and BB resistant gene(s). Besides, CRMS 31A and CRMS 32A were deployed with submergence and salinity tolerance QTLs (NRRI newsletter 2015). Grain and eating quality in hybrids is concerns which are addressed by stacking QTLs/genes for quality traits in parents. Zhenshan 97A seed parent of several hybrids in China has been stacked with QTLs of AC, GC and GT (Zhou et al. 2003); Efforts were made towards quality improvement of both the parental lines of popular indica hybrids viz. Xieyou57, using marker assisted selection for Wx locus (Ni et al. 2011). Yield enhancing QTLs yld1.1 and yld2.1. from O. rufipogon to restorer 'Ce64' (Duan et al. 2013) are successfully stacked. Hybrid sterility in inter-subspecific (indica/ *japonica*) hybrids is reported to be effectively adressed by utilizing genome editing tool 'CRISPR/Cas9' (Shen et al. 2017).

## 4.3. Screening of Rf genes in parents

Limited availability of fertility restorer system in rice makes three-line system very selective and less heterotic. Rice genotypes have fertility restorer ability can only be utilized as pollen parent in three-line hybrid breeding. Identification of genetically compatible, well combining restorers is tedious process, involve laborious test cross generation and evaluation steps. However, prior information on fertility restorer genes in the pollen parent excludes test cross steps thus make it convenient for saving time of hybrid development. Plenty of co-segregating molecular markers (tightly linked or functional markers) for fertility restorer gene(s) having functional specificity to diverse CMS systems are available (Table 3). The genic/functional markers, RM6100 and DRRM Rf3-10 of restorer gene(s) *Rf4* and *Rf3* respectively are widely utilized for screening the fertility restoration efficacy of unknown pollen parents for WA and lineage CMS well in advance (Katara et al. 2017).

## 4.4. Screening of parental lines for wide compatibility genes

Hybrid sterility is common nuisance menacing breeder to exploiting heterosis in inter-subspecific (5-10% more heterosis) hybrids. Generally, *indica* x *japonica* hybrids are sterile due to lack of wide compatibility (*WC*) between parents. It is reported that hybrid sterility in inter-subspecific crosses is mainly affected by the genes at *Sb*, *Sc*, *Sd* and *Se* (Guo et al. 2016) loci causes male sterility in  $F_1$  and the gene at *S5* locus cause female sterility in  $F_1$ . Presence of these genic region in at least one parent ensure complete fertility in resulting hybrids. These gene(s) can be assessed in advance by utilizing co-segregating markers (S5-InDel, functional marker to S5<sup>n</sup> (Priyadarshi et



al. 2017) and G02-14827 (genic marker) PSM8, PSM12 and PSM180 (linked SSR); IND19 and ID5 (indel markers) to *Sb*, *Sc*, *Sd* and *Se*, loci). Thus, it helps breeder in selection of *WC* positive parent in more predictable way which circumvents laborious test-cross and their evaluations steps.

## 4.5. Prediction of heterosis

Genetic distance and level of genetic gain/breeding value in parents are major determinant of extent of heterosis in resulting hybrid. Molecular markers help in assess the genetic diversity among parents and breeding values in progenies (through genomic selection, high density SNP genotyping) with great convenient. There are abundant STMS, SNP markers are available which can be utilized for assessment of genetic diversity/genetic distance between parents and genomic selection in progenies easily (Soni et al. 2017). Hence, this is helpful in the selection of diverse parents with maximum breeding values in turn higher heterosis or genetic gain in hybrids.

### 4.6. Determination of heterotic group and heterosis pattern

The extent of genetic variation and selection strategies are key to the success of heterosis breeding. Accurate assessment and assignment of parental lines into heterotic groups 'group of genotypes (related or unrelated) having similar combing ability and heterosis response when crossed with the genotypes of other diverse group' are fundamental prerequisite. Usually it is evaluated by combining ability analysis of parents and hybrids in multi-environment trials. However, advances in molecular marker technology have made it possible to combine information on parental pedigree and field trials with molecular marker data to detect and establish heterotic groups. Several heterotic group has been developed and utilized for three-line and two-line hybrid development in rice (Lu and Xu 2010).

### 4.7. Excavating QTLs/gene(s) responses heterosis

Omics techniques reported to have great potential in excavation of QTLs/gene(s) responses heterosis in rice. By utilizing genomics tools many QTLs/genes for several important traits has been mapped, validated and deployed in trait development in rice. The transcriptomics, an emerging technique helps in genome-scale comparisons of the transcripts of different individuals within the same species/population. It helps in understanding the level of variation for gene expression, as measured by transcript abundance that exists within plant species and between hybrids and their parents. This is useful for identification of transcript and gene *per se* involves in heterotic expression. Moreover, epigenetics, a post translational biochemical regulation of gene is found to be playing substantial role in trait expression. Individuals of the same species can have epigenetic variation in addition to genome and transcriptome content variation. A potential role for epigenetic regulation in heterosis has been proposed. It is possible for epigenetic variation to affect heterosis by creating stable epialleles that would behave similarly to the genomic or transcriptomic differences. Alternatively, hybrids may exhibit unique epigenomic states that lead to heterosis.



# 5. MAJOR CHALLENGES AND POTENTIAL RESEARCH OPPORTUNITIES

## 5.1. Major Challenges

Despite of being remunerative and varietal abundancy, HR technology could not make substantial dent in the rice farming system outside China. The following are the inherited void led poor acceptability and acreage expansion of hybrids:

**5.1.1. Lack of cytoplasmic diversity in countries outside China:** Outside of the China, WA-CMS or their lineage are commonly utilized as seed parent in more than 90% rice hybrids. Several alternative MS cytoplasmic sources such as BT-CMS, HL-CMS, CW-CMS are identified in China, but hybrid breeding programme of the other countries relied only on WA-CMS which has several inherited abnormalities. These narrowed genetics of sterile cytoplasm limits the extent heterosis exploitation and make hybrids vulnerable to many biotic and abiotic stresses.

**5.1.2. Marginal heterosis in intra-subspecific hybrids:** Two-lines and intersubspecific (*indica/japonica*) hybrids are comparatively more heterotic (5-10%) than three-lines *indica* hybrids. But owing to several inevitable difficulties in seed production of two-line hybrids and poor grain and eating quality in inter-subspecific hybrids, both could not be exploited in the countries like India who has vast climatic and food affection diversity. We are utilizing three-lines *indica* hybrids which is comparatively less heterotic hybrid breeding system giving low yields. Hence, focused and intensive research is proposed to make above said hitches be addressed in future.

**5.1.3. Poor grain and eating quality:** In hybrids, consumable parts are  $F_2$  grains, segregating for various quality traits hence very poor in quality limits its acceptability among stakeholders. Therefore, make hybrids more sustainable and popular, quality traits in hybrids needs to be addressed urgently in the country like India where people have vast category of food fondness. Hence, a strong breeding strategy for quality concern in hybrids is needs to be devised and implemented.

**5.1.4. Subtle information on QTLs/gene(s) responding heterosis:** Although heterosis, or hybrid vigour, is widely exploited in agriculture, but despite extensive investigation, complete description of its molecular underpinnings has remained elusive. It appears that there is not a single, simple explanation for heterosis. Instead, it is likely that heterosis arises in crosses between genetically distinct individuals because of a diversity of mechanisms. Hence, mining factors responding heterosis in rice will have substantial role in development and exploiting heterosis in most precise way.

**5.1.5. Inter-subspecific hybrid sterility:** Hybrid sterility is key nuisance in inter-subspecific hybrids, limiting development and commercialization of more heterotic *Indica/japonica* hybrid in rice. The sterility in hybrids (*inter-subspecific*) generally occurs due to non-functional pollens as well as sterility in female reproductive organs. It is reported that mutant of *S-i* alleles at *Sb, Sc, Sd* and *Se* loci produce sterile pollens; and mutants of *S5* locus causes sterility in female gamete. Hence, trait development for wide compatibility in either parent has great opportunity in addressing the hybrid sterility in rice.



## 5.2. Potential research opportunity

**5.2.1. Exploitation of inter-specific heterosis:** Inter-subspecific (*indica/japonica*) hybrids as discussed in earlier section are more heterotic than intra-subspecific hybrids. However, owing to hybrid sterility and poor grain quality, this genetic pool remains untapped. Grain quality of inter-subspecific hybrids proposed to be improved by utilizing parental combinations having good combining ability but similar in quality parameters, might reduce the concern of segregation for quality traits. Hybrid sterility problem in inter-subspecific hybrids can be adressed by stacking *indica* allele (*S-i*) at *Sb*, *Sc*, *Sd* and *Se* loci and the neutral allele (*S-n*) at *S5* locus in to *japonica* genetic background (Guo et al. 2016) or by silencing the *S-i* and *S5* mutant loci through genome editing tools (Shen et al. 2017).

**5.2.2. Utilization of Iso-cytoplasmic restorers:** In three-lines hybrid system, cytoplasm of CMS exerts various unwanted effect (called CMS penalty) and reduces the complete heterosis expression (up to 5-10%) in CGMS hybrids. Iso-cytoplasmic restorer is fertile transgressive segregant of CGMS hybrid, having same cytoplasm as of CMS. In combination with iso-cyto-CMS, it can normalize the fatal cyto-nuclear conflicts, hence enhances the heterosis to substantial extent. In rice, several iso-cytoplasmic restorers has been developed and utilized in hybrid rice research (Kumar et al. 2017).

**5.2.3. Out-crossing enhancement in seed parent:** Low seed producibility (1.5-2.5 t/ ha) in the CMS remains a concern, restricts seed abundancy and area expansion in India. Trait development in seed parent for out-crossing traits like stigma exertion, complete panicle exertion is important, needs to be addressed strategically. Recently, a CMS line, IR-79156A possessing more than 50% out-crossing, developed by IRRI showed seed producibility of 3.5 t/ha.

**5.2.4. Ideotype hybrid breeding:** To maximize genetic gain in rice, breeding of ideal plant type was started long back in Japan and subsequently adopted by China. Through morphological improvement and adopting inter-subspecific (*indica/japonica*) hybrid strategies, substantial progress in ideotype hybrid breeding 'super hybrid' have been achieved. China, indeed has made considerable progress and released more than 100 high yielding super hybrids (Yuan et al. 2017). Hence, inclusion of inter-subspecific quality type inbreds 'super rice' in hybrid development will have substantial impact in attaining quantum genetic gains in hybrids.

## 6. ECONOMIC IMPORTANCE

Inspite of being more cumbersome and high input intense practice, hybrid rice seed production is a profitable venture. It creates additional job opportunity (requires 100-105 more-man days) and provides more net income (around Rs. 75,000/ha net income, 70% more than the unit production cost) as compared to seed production of HYV (Rs. 13,000/ha, only 18% more than production cost) (Table 5). The market price of hybrid seed is Rs. 250-270 per kg. The farmers producing the hybrid seed get only Rs. 80-90 per kg. In case of low production (<5 quintal/acre) farmers get minimum Rs. 45,000 as compensation from seed production agencies.



			Cost/income	(in Rs.)
Item	Quantity/Nu	umber (per hectare)	Hybrid seed	HYV
Seed cost	Male	5 kg @ Rs. 50/kg	250	2,000
	Female	15 kg @ Rs.400/kg	3,000	Nil
Labour cos	t	250/145 @ Rs. 200/labour/day	50,000	29,000
FYM and f	ertilizer cost	N:P:K (100:50:50) (based on market price)	5,400	5,400
Irrigation		18-20 Irrigation (weekly) @Rs.1500/ha/ 30,000	30,000	
Gibberellic	acid		2,000	Nil
Others			15,000	10,000
Total cost			1,05,650	76,400
Average production			2.0 t/ha	4.5
Gross incom	me	*Price @ Rs. 90/kg and Rs. 20/kg	1,80,000	90,000
Net income	;		74,350	13,600

### Table 5. Cost analysis of hybrid rice seed

\* Price of seed is the price given to the farmer.

## 7. WAY FORWARD

Under changing climatic and agriculture scenario rice hybrid is likely to face stiff competition to sustain in future. Despite having great potential to enhance production and productivity, it has not been adopted on large scale as was expected. This is due to several constraints like lack of acceptability of hybrids in some regions such as southern India, due to region specific grain quality requirement. Moderate (15–20%) yield advantage in hybrids is not economically very attractive and there is a need to increase the magnitude of heterosis further. Lower market price offered for the hybrid rice produce by millers/traders is acting as a deterrent for many farmers to take up hybrid rice cultivation. Higher seed cost is another restrain for large scale adoption and hence there is a need to enhance the seed yield in hybrid rice seed production plots. Efforts for creating awareness and for technology transfer were inadequate in initial stages. Involvement of public sector seed corporations in large scale seed production has been less than expected. Hybrids rice for aerobic/upland, boro season and long duration hybrids for shallow lowland conditions to be developed. Most of the constraints mentioned above are being addressed with right earnestness through the on-going research projects and transfer of technology efforts.

### References

- Basavaraj SH, Singh VK, Singh A, Singh AK (2010) Marker-assisted improvement of bacterial blight resistance in parental lines of Pusa RH10, a superfine grain aromatic rice hybrid. Molecular Breeding 26(2): 293-305.
- Behera L, Patra BC, Sahoo RK, Nanda A, Patnaik A, Singh ON (2012) Assessment of genetic diversity in medicinal rice using microsatellite markers. Australian Journal of Crop Sciences 6(9):1369-76.



- Chen S, Lin XH, Xu CG and Zhang Q (2014) Improvement of Bacterial Blight Resistance of 'Minghui 63', an Elite Restorer Line of Hybrid Rice, by Molecular Marker-Assisted Selection. Crop Science 40:239–244.
- Dash AK, Rao RN, Rao GJN, Verma RL, Katara JL, Mukherjee AK, Singh ON and Bagchi TB (2016) Phenotypic and Marker-Assisted Genetic Enhancement of Parental Lines of Rajalaxmi, an Elite Rice Hybrid. Frontier in Plant Science 7: 1005.
- Duan MJ, Sun ZZ and Shu LP (2013) Genetic analysis of an elite super-hybrid rice parent using high-density SNP markers. Rice 6(1):21-36.
- Fujii S, Toriyama K (2005) Molecular mapping of the fertility restorer gene for ms-CW-type cytoplasmic male sterility of rice. Theoretical and Applied Genetics 111:696-701.
- Guo J, Xu X, Li W, Zhu W, Zhu H, Liu Z, Luan X, Dai Z, Liu G, Zhang Z, Zeng R, Tang G, Fu X, Wang S and Zhang G (2016) Overcoming inter-subspecific hybrid sterility in rice by developing indica-compatible japonica lines. Scientific Report 6:268-278.
- Hu J, Wang K, Huang W, Liu G, Gao Y (2012) The rice pentatricopeptide repeat protein *RF5* restores fertility in Honglian cytoplasmic male-sterile lines via a complex with the glycine rich protein GRP162. Plant Cell 24:109-22.
- Igarashi K, Kazama T and Toriyama K (2016) A Gene Encoding Pentatricopeptide Repeat Protein Partially Restores Fertility in RT98-Type Cytoplasmic Male-Sterile Rice. Plant Cell Physiology 57(10):2187-2193.
- Itabashi E, Iwata N, Fujii S, Kazama T, Toriyama K (2011) The fertility restorer gene, *Rf2* for Lead Rice-type cytoplasmic male sterility of rice encodes a mitochondrial glycine-rich protein. The Plant Journal 65:359-367.
- Jonse JW (1926) Hybrid vigour in rice. Journal of American Society of Agronomy 18: 423-428.
- Katara JL, Verma RL, Nayak D, Umakanta N, Ray S, Subudhi H, Behera L, Samantaray S, Singh ON, Mohapatra T (2017) Frequency and fertility restoration efficiency of *Rf3* and *Rf4* genes in Indian rice. Plant Breeding, 136:74-82.
- Kumar A, Bhowmick PK, Singh VJ, Malik M, Gupta AK, Seth R, Nagarajan M, Krishnan SG, Singh AK (2017) Marker-assisted identification of restorer gene(s) in iso-cytoplasmic restorer lines of WA cytoplasm in rice and assessment of their fertility restoration potential across environments. Physiology and Molecular Biology of Plants 23(4):891-909.
- Liu XQ, Xu EX, Li SQ, Hu J, Huang JY, Yang DC, Li YS, Zhu YG (2004) Inheritance and molecular mapping of two fertility-restoring loci for Honglian gametophytic cytoplasmic male sterility in rice (*Oryza sativa* L.). Molecular Genetics and Genomics 271:586-594.
- Liu, W, Wang, FM and Jin, S (2008) Improvement of rice blast resistance in TGMS line by pyramiding of Pi1 and Pi2 through molecular marker-assisted selection. Acta Agronomica Sinica 34: 1128-36.
- Lu ZM and Xu B (2010) On significance of heterotic group theory in hybrid rice breeding. Rice Science 17:94-98.
- Ni D, Zhang S, Chen S, Xu Y, Li L, Li H and Yang J (2011). Improving cooking and eating quality of Xieyou57, an elite indica hybrid rice, by marker-assisted selection of the Wx locus. Euphytica 179(2): 355-362.
- Okazaki M, Kazama T, Murata H, Motomura K and Toriyama K (2013). Whole Mitochondrial Genome Sequencing and Transcriptional Analysis to Uncover an RT102-Type Cytoplasmic Male Sterility-Associated Candidate Gene Derived from *Oryza rufipogon*. Plant Cell Physiology 54(9):1560-1568.



- Priyadarshi R, Hari Prasad SA, Singh AK, Khandekar D, Ulaganathan K and Shenoy V (2017) Molecular stacking of wide compatibility gene, S5 n and elongated uppermost internode (*eui*) gene into IR 58025B, an elite maintainer line of rice. Journal of Plant Biochemistry and Biotechnology 26(4): 425-435.
- Rao GS, Deveshwar P, Sharma M, Kapoor S and Rao KV (2018) Evolvement of transgenic malesterility and fertility-restoration system in rice for production of hybrid varieties. Plant Molecular Biology 96:35-51.
- Sampath S, Mohanty HK (1954) Cytology of semi-sterile rice hybrid. Current Science 23:182-183.
- Shen R, Wang L, Liu X, Wu J, Jin W, Zhao X, Xie X, Zhu Q, Tang H, Li Q, Chen L, Liu YG (2017) Genomic structural variation-mediated allelic suppression causes hybrid male sterility in rice. Nature Communications 8:1310-1320.
- Soni SK, Yadav VK, Bhadana VP, Yadav MC and Sundaram RM (2017) Prediction of heterosis using hypervariable SSR markers in tropical japonica x indica rice hybrids (*Oryza sativa* L). International Journal of Current Microbiology and Applied Sciences 6 (10):1419-1427.
- Tang H, Zheng X, Li C, Xie X, Chen Y, Chen L, Zhao X, Zhou J, Ye S, Guo J, Liu YG (2017). Multistep formation, evolution, and functionalization of new cytoplasmic male sterility genes in the plant mitochondrial genomes. Cell Research 27:130-146.
- Tao C, Ming L, Zya Z, Zhen Z, Ling Z, Qing Y, Shu Y, Xin Y, Cai W (2013) Development and Application of a Functional Marker Associated with Fertility restoring Gene for BT-type Cytoplasmic Male Sterility (CMS) in japonica Rice. Chin Journal of Rice Science 27(3):259-264.
- Toriyama and Kazama (2016) Development of Cytoplasmic Male Sterile IR24 and IR64 using CW-CMS/*Rf17* System. Rice 9:22-26.
- Verma RL, Singh S, Singh P, Kumar V, Singh SP, Singh S, Samantaray S and Singh ON (2017) Genetic purity assessment of indica rice hybrids through DNA fingerprinting and grow out test. Journal of environmental Biology 38(6):1321-1331.
- Wang ZH, Zou YJ, Li XY, Zhang QY, Chen LT, Wu H (2006) Cytoplasmic male sterility of rice with Boro II cytoplasm is caused by a cytotoxic peptide and is restored by two related PPR motif genes via distinct modes of mRNA silencing. Plant Cell 18:676-87.
- Wang K, Gao F, Ji Y, Liu Y and Dan Z (2013) ORFH79 impairs mitochondrial function via interaction with a subunit of electron transport chain complex III in Honglian cytoplasmic male sterile rice. New Phytologist 198: 408-418.
- Yuan LP (2017) Progress in super-hybrid rice breeding. The Crop Journal 5:100-102.
- Zhang H, Xu C, He Y, Zong J and Yang X (2013) Mutation in CSA creates a new photoperiodsensitive genic male sterile line applicable for hybrid rice seed production. Proceeding National Academy of Science, USA 110:76-81.
- Zhou PH, Tan YF, He YQ and Zhang Q (2003) Simultaneous improvement for four quality traits of Zhenshan 97, an elite parent of hybrid rice, by molecular marker-assisted selection. Theor atical and Applied Genetics 106:326-331.
- Zhou H, He M, Li1 Z, Chen L, Huang Z, Zheng S, Zhu L, Ni E, Jiang D, Zhao B and Zhuang C (2016) Development of Commercial Thermo-Sensitive Genic Male Sterile Rice Accelerates Hybrid Rice Breeding Using the CRISPR/Cas9- mediated TMS5 Editing System. Scientific Report 6:373-395.



# New Generation Rice for Breaking Yield Ceiling

## SK Dash, P Swain, LK Bose, R Sah, M Chakraborti, N Umakanta, K Chakraborty, M Azharudheen TP, S Lenka, HN Subudhi, J Meher, S Sarkar, A Anandan, M Kar, S Munda, SK Pradhan, L Behera and ON Singh

## SUMMARY

A breakthrough in yield ceiling in rice is warranted in view of increasing competition for resources. Ideotype/ New Plant Type/ New Generation Rice is one of the potential approach based on tailoring a plant architecture with incorporation of efficient traits for harnessing light and nutrients for optimum biomass (source) and grain yield (sink). Initial breakthrough in yield improvement was accomplished by introduction of dwarfing genes during sixties from *japonica*. Subsequently, rice improvement focus was shifted for augmentation of stress resistance and quality in shorter growth duration; hence efforts towards yield increment were not much rewarding. 'New Plant Type' approach tried to improve the productive features from tropical japonica with heavy panicles, high grain number and shy tillers. Subsequently, 'Chinese Super Rice' concept further modified it with incorporation of erect, long and wide leaves with less panicle height for increasing biomass. Recent discoveries on mapping of QTLs/genes for yield attributing and stress tolerant traits improves the probability of fine tuning in the existing super/popular rice cultures, for trait specific complementation through marker assisted selection and transgenic means from diverse sources, including wild rice. Similarly, manipulation of some physiological process can also help for improving overall performance. Over and above there should be standardized management practices for full realization of yield potential.

## **1. INTRODUCTION**

The Green Revolution in mid sixties increased the rice production of the world remarkably. However, a ceiling of grain yield potentiality was mostly reported in semidwarf inbred *indicas* since release of IR 8 (Peng et al. 2008), despite of significant achievement in yield stability, increased per day productivity and improved grain quality (Aggarwal et al.1996). A breakthrough in productivity barrier is warranted in view of increasing competition for water and other resources because of increased population coupled with higher industrialization, urbanization and diversion of agricultural land.

There are several available options, viz., Hybrid Rice, New Plant Type/New Generation Rice (NGR) and  $C_4$  Rice. However, there is significant progress for the first two categories only. Hybrid rice basically targets exploitation of heterosis resulting from heterozygous  $F_1$  from two different inbreeds, whereas, NPT focuses on tailoring a novel plant architecture with incorporation of traits supposed to be most efficient



for harnessing light and nutrients for optimum biomass(source) and its competent and productive partition in to the grain yield (sink). Commercial success has been achieved in China and India in respect of hybrid rice utilizing three line and two line approaches and has clearly demonstrated the potential of this technology. However, the success of hybrids mostly depends on the potentiality of restorer lines. Therefore, a very high yielding restorer coupled with other critical requirements are the key for success of hybrid rice. Furthermore, a super yielding genotype would greatly help this technology to attend new heights of grain yield.

The C<sub>3</sub> photosynthetic pathway is less efficient than C<sub>4</sub> pathway. The goal is to transform the existing photosynthetic mechanism to a higher capacity one. Taking a lesson from evolution and converting a plant from C<sub>3</sub> to C<sub>4</sub> would involve a rearrangement of cellular structures within the leaves and more efficient expression of various enzymes related to the photosynthetic process. However, all the components for C<sub>4</sub> photosynthesis already exist in the rice plant, but they are distributed differently and are not as active. The current approach targets to identify the genes responsible to install C<sub>4</sub> photosynthesis through different approaches, including genomic and transcriptional. However, it may take some time to have some tangible achievements.

In all these approaches the basic aim is enhancement of grain yield potential. Evans (1993) defined the term "yield potential" as the yield of a variety when grown in environments to which it is most adopted, with nutrients and water non-limiting and the pests and diseases and stresses effectively controlled. Yield potential could be increased with enhancement of morpho-physiological traits by modifying the plant design and harnessing better genetic gain from transgressive segregants or hybrids. The objective of this chapter is to discuss the recent developments towards developing high yielding varieties for breaking yield ceiling, in the light of preference of farmers and consumers, taking into account the chronological research efforts for yield enhancement.

# 2. IDEOTYPE CONCEPT

The NGR discussed here basically stands on ideotype concept or approach of crop improvement. Ideotype (ideal plant type) is defined as "a biological model, which is expected to perform or behave in a predictable manner within defined environment" (Donald 1968). Again a Crop ideotype is defined as "An idealized plant type with a specific combination of characteristics favourable for photosynthesis, growth and grain production based on knowledge of plant and crop physiology and morphology". There are different types of ideotype conceptualized (Singh 2002) as listed:

*Isolation ideotype:* It is the model plant type that performs best when the plants are space planted. In rice, it is lax, free tillering and leafy. A spreading plant is able to explore environment as fully as possible. It is unlikely to perform well at crop densities.

*Competition ideotype:* This performs well in genetically heterogeneous population, such as, the segregating generation of crosses and performs better while competing



with weeds. In rice it is relatively tall, leafy, free tillering plant that is able to shade its less aggressive neighboursand, thereby, gain larger share of nutrition and water. In annual seed crop like rice, the seed size, speed of germination and root characters also matters.

*Communal/crop ideotype:* This performs best at commercial crop densities because it is a poor competitor. It performs well when it is surrounds by plants of same form. But it performs less when surrounds with plants of other form, e.g., competition ideotype and isolation. In rice, a communal or crop ideotype can be able to survive in the highly competitive situation. The concept of 'weak competitor' is the central theme of this ideotype. Different set of characters have been conceptualized in rice by different workers.

Tsunoda (1962) correlated yield capacity and yield response to nitrogen using different rice plant type and could discover that varieties with superior yielding ability and higher responsiveness to nitrogen were closer to short sturdy stems and erect, short, narrow, thick, and dark green leaves.

The close association between certain morphological traits and yielding ability in response to N led to the "plant type concept" as a guide for breeding improved varieties (Yoshida 1972). In rice, ideal plant type was hypothesized as early as 1962. This ideal plant type was designed to maximize solar radiation interception, minimize lodging and response to inputs

## 3. STATUS OF RESEARCH

### 3.1. International works

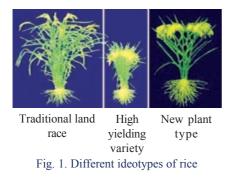
### 3.1.1. Conventional rice improvement

3.1.1.1. Quantum jump in rice productivity with dwarfing gene: A major breakthrough in yield improvement was accomplished by introduction of dwarfing genes. During 1956, dwarfing gene was in used in breeding from local landrace Ai-zi-zhan to develop variety Guang-chang-ai, released during 1959 (Huang 2001) in China. In 1962, rice breeders of IRRI took initiative to introduce dwarfing genes from Taiwanese varieties such as Dee-geo-woo-gen, Taichung Native 1, and I-geo-tse to tropical tall land races. In 1966, IR 8, the first semi-dwarf, high-yielding modern rice variety, was released for the tropical irrigated lowlands (Khush et al. 2001). The development of IR8 increased the yield potential of the irrigated rice varieties in tropics from 6 to 10 t ha<sup>-1</sup> (Chandler1982). The focus in the entire rice breeding programme was to increase in yield potential. Tropical varieties of enormous yielding capacity, viz., Jaya in India and BG 90-2 in Sri Lanka were developed. In Korea, Tongil-type rice varieties were developed in 1971 from a japanica/ indica cross (Chung and Heu1980), showed a 30% yield increment compared with *japonica* varieties. Morphologically, Tongil varieties were characterized by medium-long and erect leaves, thick leaf sheaths and culms, short plant height but relatively long panicles, open plant shape with lodging resistance. Similarly, during 1982, indica/japonica hybridization by Japanese breeders



for a targeted super-high-yielding rice development, resulted in several promising super-high-yielding cultivars such as Akenohoshi and Akichikarawith heavy panicle along with large number of spikelet per panicle (Wang et al.1997).

The dwarf plant type was discovered to be due to 'sd1' gene in Dee-Geo-Woo-Gen and others genotypes and was a landmark in development high yielding variety, which resulted in green revolution. This resulted in remarkable change in plant



architecture,viz., dwarf height, high tillering, sturdy stem, dark green and erect leaves (Fig. 1). It was further coupled with photo-insensitiveness and fertilizer responsiveness which enhanced its efficiency to have a productivity of 10 t ha<sup>-1</sup> during dry season at Philippines. Further, it was contributed by the diversity of indica and japonica. Sufficient genetic distance supposedly resulted in heterosis and subsequent potential transgressive segregants with accumulation of the traits

suitable for higher grain yield eliminating the necessary bottlenecks prevailing thereof.

Subsequent plant breeding efforts towards yield increment were not much rewarding. This might be me shifting of focus towards maintenance of productivity in prevailing biotic stress situation by augmenting disease and pest resistance, superior grain quality, and shorter growth duration. Beachell, Khush and the IRRI team could succeed in developing one of the highly popular variety and extensively grown, IR36, in the 1970s. Lately, Khush and team could improve upon it with development of IR72, with productivity potential equivalent to IR8 but have shorter growth duration and improved resistance to a number of important rice diseases and insect pests. When adjusted for earlier maturity, the yield potential of IR72 is 5-10% greater than IR8 on a yield per day basis. However, stagnant yield potential of semi-dwarf *indica* inbreds observed since the release of IR8 (Peng et al.2008).

3.1.1.2. New plant type approach: While critically analyzing the causes of yield stagnation, physiologists hypothesized that the stagnation might be the result of the plant architecture having high tillering and small panicles. Several unproductive tillers along with lodging susceptibility that supposedly limit sink size limiting yield enhancement. Furthermore, these have excessive leaf area that may cause mutual shading and a reduction in canopy photosynthesis and sink size, especially when grown under direct seeded conditions (Dingkuhnet al.1991).

Several approaches were there for raising yield ceiling in irrigated ecosystem, and New Plant Type (NPT) breeding to break yield ceiling is one of the potential and farmers' friendly approach conceptualized by IRRI scientists (Peng et al.2008). The objective was to further modify the present high-yielding plant type to support a significant increase in yield potential. The basic plan was conceptualized on the basis



of ideotype approach along with simulation modeling taking into view the framework proposed by crop physiologists.

Simulation models could foresee the possibility of increase in yield potential to the tune of 25% by alternation of the following physiological and morphological traits of the earlier plant type (Dingkuhn et al. 1991):

- A plant type with lesser tillers and high leaf growth during early vegetative stage because this stage is mainly responsible for higher tillers.
- Retarded leaf expansion and more foliar N concentration during late vegetative and reproductive growth.
- An abrupt reduction of the vertical N concentration gradient in the leaf canopy with a large chunk of total leaf N in the top leaves.
- Higher carbohydrate storage capacity in stems, and
- A greater reproductive sink capacity and an extended grain-filling period.

The NPT hypothesized for another quantum jump with the rationale that grain yield is an outcome of total dry matter and harvest index (HI). Harvest index could be strengthened by enhancing sink capacity. However, augmenting both of these could boost the productivity. The choice for proper traits to develop an ideal plant type for the irrigated lowland turned up from different outlooks. It emphasized combining heavy panicle with 200-250 grains with proportionately less tiller in short statured plants (90-100cm). The stem should be sturdy to resist lodging and leaves should be erect, thick and deep green to support high net assimilation rate. Moreover, it should have high HI and deep and vigorous root system. There should be sufficient field tolerance to major disease and pest. The genotypes with enhanced yield potential and better responsiveness to N administered, had short sturdy stem with erect, short, narrow, thick and dark green leaves. The "Plant Type Concept" focused mostly on modification of certain morpho-physiological traits leading to higher grain yield in response to nitrogen as guiding principle for breeding.

New Plant Type was designed to maximize solar radiation interception, minimize lodging and high response to inputs with a view to improve biomass and harvest index that paves the way for high grain yield. The target was to develop a plant type within 8-10 years with a modest yield increment up to 30-50% than the existing semi dwarf varieties in tropical environments during the dry season (Peng et al. 2008). With this concept, donors with large panicle, thick stem, short stature and low tillering types, *bulu* or *javanica* (*Tropical japonica*) type germplasm from Indonesia, Malasyia, Thailand, Mynamar, Laos, Vietnam and The Phillippines were selected and hybridization was done. Large scale hybridization and selections (2000 crosses and 100,000 pedigree lines) were done. First Generation NPTs were selected with large panicle, few unproductive tillers and lodging resistance and extensive yield trials were conducted to assess the performances. However, the population performance was not satisfactory and grain yield was not encouraging. Critical analysis of this disappointing result could find that there was low biomass production due to reduction



in tiller number m<sup>-2</sup>, less crop growth rate (CGR) along with poor translocation of assimilates during grain filling from the biomass accumulated at pre-flowering, in comparision to *indica* varieties. Similarly, other major possible causes assigned were poor grain filling, which might be due to less biomass, lack of epical dominance, compact panicles, limited number of large vascular bundles and early leaf senescence (Peng et al.2008) etc. These were coupled with susceptibility to major diseases and pests and were having poor grain quality, hence, could not be released to farmers' field.

Although partially successful, it provided a strong foundation for further research on yield increment utilizing *tropical japonicas*. The promising 1st generation NPTs were hybridized with elite *indicas* in order to increase the effective tiller numbers but reduced the panicle size. The reduced grain number with the same panicle size made the panicle less compact, and in turn, increased the grain filling in the second generation NPTs. Moreover, accumulation of more genome from adapted varieties enhanced the quality of grains and disease and pest resistance. With necessary trait specific augmentation few lines could outyield IR 72; one among them, IR72967-12-2-3 produced10.16 t/ha, higher to *indica* check PSBRc52. Few of them could be released successfully in Philippines and China (Peng 2008).

*3.1.1.3. China's Super rice:* In china, in addition to the traits proposed earlier, early vigour was proposed to have more effect on high yield with development of bushy-type varieties (Huang 2001). These varieties are tolerant to shading and high plant density, and were widely grown in southern China. Supplementary advantage in yield potential was proposed by Yang et al.(1996) from a combination of improvement in plant type and use of growth vigor. With the influence of IRRI's NPT programme and super high yielding hybrid rice combination , which could record a 17.1 tha<sup>-1</sup> yield, a' super' hybrid rice initiative was started in 1998 by Prof. L. P. Yuan. Here, the strategy was to combine an ideotype approach with the use of inter-sub-specific heterosis. The ideotype was reflected in the following traits (Peng 2008):

- Tall erect leaf canopy: The primary three leave blades from the top should be erect and long and wide (2 cm) to have a higher leaf area. Erect leave will facilitate reception of light in both sides and avoid mutual shading. The Flag-leaf should be long (50 cm) followed by still longer second and third leaves (55 cm each). All three leaves should be on the top of panicle height. Leaves should remain erect until maturity and the angles of the top three leaves should be ~ 5°, 10°, and 20°, respectively. The leaf should be stiff, narrow, V-shaped and thick (specific leaf weight of 55 g m<sup>-2</sup>) to have stay green character and delayed senescence and enhanced photosynthetic efficiency. Moreover, leaf area index of these three leaves should be high (>6.0).
- Moderate tillering capacity: Instead of low tiller here moderate tiller number (8-10 tillers plant<sup>-1</sup> or 270-300 m<sup>-2</sup>) has been proposed. The plant height should be semi-dwarf with at least 100cm and the panicle height should be 60 cm from the soil surface during maturity.



- Large panicle: The panicle should moderately heavy with 5.0g panicle<sup>-1</sup>. With about 300 panicles m<sup>-2</sup> the theoretical yield potential is 15 t ha<sup>-1</sup>.
- High HI: The harvest index should be around 0.55 or nearer to that. Harvest index of ~0.5 requires more of biomass. An increased plant height could be an option on morphological point of view. However, a tall plant is prone to lodging, a potential hazard for yield loss which needs avoidance.

Physiologists advocates thicker and sturdy culm, which again decreases HI and reduces the chance of super grain yield. In this context, this model of longer and thicker top three leaves provides a plausible solution for higher biomass, HI and resistance to lodging (Fig. 2). This is again in contrast to the IRRI's new plant type where short and sturdy culm was proposed.

During 1998–2005, several super rice hybrids were commercially released matching to the model conceptualized. However, two such varieties, viz., Xieyou 9308 and Liangyoupeijiu could be popular because of their higher yield and superior grain quality. Xieyou 9308 was an inter-sub specific hybrid produced 11.53 t ha<sup>-1</sup>in



Fig. 2. Plant type of China's supper rice

an on-farm demonstration experiment, with 17.5% higher productivity than hybrid check. Similarly, high grain yield to the tune of 12.11 tha<sup>-1</sup> was recorded by Liangyoupeijiu (inter-subspecific hybrid) in Hunan province of China during 2000 and it outyielded the hybrid check by 8–15% in farmers' fields (Peng et al. 2008). The high yield in these cases was associated with higher LAD before heading, greater biomass accumulation before heading, larger number of grains, and more translocation of carbohydrates from the vegetative organ to the panicle during the grain-filling period.

3.1.1.4. Similarities of IRRI's NPT design and China's "super" hybrid: Both NPT of IRRI and super rice plant type of China emphasized large and heavy panicles, reduced tillering capacity, and improved lodging resistance. It was expected that harvest index could be improved with increased sink size and few unproductive tillers. Other common traits are erect-leaf canopy and slightly increased plant height in order to increase biomass production. The initial strategy for the NPT at IRRI was incorporation of genes for large panicles and sturdy stems from TJ germplasm followed by crossing the improved TJ with elite *indica* varieties to produce an intermediate plant type. In contrast, "super" hybrid rice (two-line or three-line), proposed an intermediate type between *indica* and *japonica* with an indica parent in order to use inter-sub-specific heterosis.

Plant type of "super" hybrid rice, panicles are kept inside the leaf canopy by increasing the distance between panicle height and plant height. This trait was not clearly defined in IRRI's NPT design because an IRRI physiologist discovered the benefit of reducing panicle height for improving canopy photosynthesis and yield



potential only in mid-1990s. The distance between panicle height and plant height can be increased by either reducing panicle height or increasing plant height: used in developing "super" hybrid rice. However, super hybrid had more focus on the top three leaves.

*3.1.1.5. Green super rice:* Rice cultivars that can produce high and stable yield with fewer inputs (water, fertilizers and pesticides), known as green super rice (GSR). Thus, GSR varieties are climate-smart and can help farmers protect the environment and themselves (Li and Ali 2016).

GSR was supposedly developed by utilizing more than 250 promising rice varieties and hybrids that are adapted basically to different stress situations, viz., drought and low input stress with less inorganic fertilizer and no pesticide and with quick establishment rates so that it could well compete and overcome the weeds and require less herbicide, thus causing less harm to environment and would be sustainable.

In the past, breeders at IRRI used only three recurrent parents, IR64, Teqing, and IR68552-55-3-2, a new plant type variety, backcrossed with 205 donor parents. However, the GSR concept, which was conceived by the China National Rice Molecular Breeding Network, used 46 recurrent parents. Crosses were made with 500 donors, resulting in a bigger pool of available genes. Subsequently, screening was done in early generations of backcross bulk populations ( $BC_2F_2$ ) for different traits supposed to be important under different biotic and abiotic stress situation, viz.,traits such as drought, salinity, flooding, and phosphorus and zinc deficiency tolerance from a very large collection of different types of rice. The promising transgressive segregants that exceed the performance range of their parents under extreme conditions were selected.

Rather than focusing on developing one variety for all, GSR can be custom made to fit any target ecosystem. For example, GSR varieties can grow rapidly to compete strongly with weeds. Because they establish themselves much faster than the weeds, so herbicide requirement is reduced. Similarly, the project claims to have developed drought-tolerant GSR lines in IR64 background, i.e., IR83142-B-19-B, which performs better than Sahbhagidhan under drought and zero-input conditions (no fertilizers and no pesticides, and only one manual weeding) (Reyes 2009).

### 3.1.2. Biotechnological approach

High yield is an unending theme pursued by rice researchers. Breeding for super rice using molecular tools could effectively supplement empirical conventional approach. Grain yield is a complex phenomenon which is contributed by three major yield attributing traits, viz., Number of panicles per plant (NPP), Number of grains per panicle (NGP) and Grain weight (GW). NPP is dependent on the ability of the plant to produce tillers. NGP depends on the number of spikelet per panicle, number of primary and secondary branches and spikelet fertility. Similarly, GW is largely determined by grain size and seed weight. Many yield related genes/QTLs have been identified in rice, which are being utilized to improve yield potential through molecular breeding approach.



*3.1.2.1. Identification of QTLs/genes for grain yield:* Recently, many yield-related genes/QTLs have been identified and cloned in rice. A comprehensive list of these gens/QTLs related to respective trait/traits is presented in Table 1. Several scientists had reported that out of the many agronomic traits, grain weight is highly heritable and can be improved through marker assisted selection than other yield related traits. Among these traits, panicle and grain architecture supposed to be the maximum contributor for grain yield. For such traits many genes were identified either from mutant, or from homologues or identified as locus with MSU-ID. Some of the genes/QTLs independently govern one or the other component traits of grain yield. However, many of them also show pleiotropic gene action. The actual genetic gain in terms of grain yield would depend on the judicious combinations of pyramiding or stacking of these genes/QTLs in a particular genetic background.

Table 1.Genes/QTLs for rice yield traits useful for breeding super rice (adapted from Ying et al.2014 and Hirano et al. 2017).

Trait/combination of traits	Genes (identified from mutant)	Genes (identified from homologues)	QTLs (identified as locus with MSU-ID)
Number of panicles per plant	D27, D10, D14, D17/ HTD1, D3MOC1	-	-
Number of grains per panicle	LOG, LP, SP1	-	Gn1a, Hd1, Ghd7, Ghd8/DTH8, EHD1, DEP1
Number of panicles per plant, number of grains per panicle	LAX1, APO2, DEP2, FZP	-	OsSPL14, PROG1.qGY2-1
Grain weight, grain size	BRD1, SRS3, SG1SRS5	PGL2, PGL1, APG	TGW6, GW2, GS3, GL3.1/qGL3, GS5, qSW5/GW5, GW8
Grain weight, grain filling	GIF1, FLO2, HGW	-	
Culm strength, number of grains per panicle	-	-	APO1 (SCM2), OsTB1 (SCM3)

3.1.2.2.Marker-assisted selection (MAS): Marker-aided selection (MAS) has not yet been extensively used as a part of the regular breeding programme for yield enhancement. However, few scientists have utilized this approach for improvement of various traits in *Indica x Japonica* derivatives for breaking yield ceiling. The MAS techniques uses tightly linked molecular markers to target the gene of interest. Using combination of conventional and molecular breeding techniques, Wang et al.(2008) and Yao et al. (2010) successfully pyramided important genes in several varieties including Nanjing 46, Nanjing 5055 and Nanjing 9108. The gene for dense and erect panicle-1 (*Dep1*) was used to develop NILs (Nanhui 602 x DW 135) through backcrossing. Similarly, for grain size and exterior quality of seed, pyramiding was done in the genetic background of Huajingxian 74 by Yang et al. (2010) and Wang et al. (2012) effectively with *GS3* and *GW8* genes, governing grain length. Bacterial



blight and blast resistance genes were incorporated in two restorer lines, Zhonghui 8006 and Zhonghui 218. These lines were used for development of series of super rice hybrids. For improvement of grain yield, the *yld 1.1* (linked marker RM 5) and *yld2.1* (linked marker RG 256) QTLs were reported. The MAS technique was also utilized for transferring grain length and width using *GW6* gene from Baodali (*japonica* variety) into an *indica* recurrent parent 9311 and a *japonica* variety Zhonghua 11 (ZH11) using MABB. Three improved ZH11-*GW6* lines were obtained which showed more than 30% increase in grain weight and about 7% increase in grain yield. Seed plumpness of these three lines were improved synchronously because the three ZH11-*GW6* lines contained *GIF1* (*Grain Incomplete Filling 1*), which is a dominant grain filling gene. Thus, MAS will be a useful option for rapid utilization of genetic resource in super rice breeding.

3.1.2.3. Transgenic approach for vield improvement: Conventional breeding may be lengthy and associated with linkage drags/vield penalty. In this context, geneticallymodified (GM) or transgenic technology has been shown as an alternative to the conventional breeding approach. Unlike the former, the latter provides target specific or limited changes in genetic materials that are well defined and to be done in a short period of time. There have been several reports of transgenic plants but limited success for higher yield (Paul et al. 2018). In rice, Lu et al. (2015) reported that altered expression of OsPIN5b which encoded an endoplasmic reticulum (ER)-localized protein that participates in auxin homeostasis, transport and distribution in vivo, which results in higher tiller number, more vigorous root system, longer panicles and thereby improving simultaneously plant architecture as well as yield potential. Liu et al.(2015) also developed the GM rice by over-expressing BG1 gene that significantly increased grain size by increasing sensitivities to both auxin and N-1-naphthylphthalamic acid, an auxin transport inhibitor and hence improved rice plant productivity. Another GM rice had been developed by Zhang et al. (2013) through overexpression of the rice micro RNA (miRNA) OsmiR397 that resulted enlargement of grain size and more panicle branching leading to an increase in overall grain yield of up to 25% in a field trial. Therefore, this approach could be integrated with conventional approach for overall rice improvement.

*3.1.2.4. Doubled haploid breeding:* It is an important technique for quick fixation of homozygosity and shortening the breeding cycle in varietal improvement. This approach, not only increases the selection efficiency but also allows early expression of recessive genes. In conventional breeding, the early segregating generation population involves variable attributable to both additive and non-additive genetic effects whereas DH lines exhibit variation only of additive genetic nature including additive type of epistasis which can be easily fixed through a single cycle of selection. The detail of this is available in Chapter 1.10 for reference.

### 3.1.3.Other/novel approaches

*3.1.3.1.Wild ancestors of rice in yield improvement:* Narrow genetic base in cultivated rice is caused by factors such as monophyletic origin, genetic bottlenecks, and repetitive use of elite breeding lines and is one of the major factor limiting genetic improvement of cultivars. Therefore, it is a necessary to use the diversity arising from wild relatives.



Wild species are important sources of naturally occurring diverse alleles for further yield improvement. The exploitation of vast genetic resources available in the genus Oryza could be potential area of research for further improvement. In the past, wild species were often used as a source of insect and pest resistance, but were rarely used to improve complex traits such as yield (Bose 2005). However, evidence from advanced backcross quantitative trait locus (AB-QTL) analysis followed by molecular mapping studies showed that phenotypically poor wild species can contribute genes for improving yield and such loci can be mapped after introgression into elite cultivars (Tanksley and McCouch1997). Wild rice species are more diverse than cultivated varieties (Swamy and Sarla 2008). Among the wild accessions, genetically moderate distant accessions are the best choice as donor parents, because they contain less undesirable alleles than distant accessions. In rice, yield and yield-related QTLs have been identified from three wild rice species such as O. rufipogon, O. glumaepatula and O. grandiglumis using AB-QTL strategy. O. rufipogon, a perennial wild progenitor of Asian cultivated rice, is used for mapping of QTLs for yield and grain quality, and identification of other related traits under different genetic backgrounds (Xie et al.2008).

Several plant traits directly or indirectly affect rice grain yield including days to heading and maturity, plant height, panicle length, number of panicles per plant, spikelets per panicle, grains per panicle, seed set, grain weight, grain size and shape, and shattering. Yield improvement can be achieved as a result of the vast allelic diversity for these traits found in interspecific populations, especially number of grains per panicle which has proven to have the greatest relevance for rice breeding programs (Tian et al 2006). Modern rice varieties are developed after an extensive selection process to improve a few targeted traits related to cultivation and end-use quality but primarily those associated with yield components, such as resistance to shattering, compact growth habit and improved seed germination (Tanksley and McCouch 1997). This prolonged breeding procedure can lead to a reduction in the genetic variability found in modern cultivated rice. Thus identifying genetic sources for agronomically important traits from wild Oryza species and introgressing them into cultivated rice is desirable and necessary. Although wild Oryza species are inferior in grain yield, especially when compared to cultivated rice, transgressive segregation from a cross between cultivated rice and a wild Oryza species, especially the ancestral species, O. rufipogon and O. nivara, revealed the presence of favorable alleles from the wild parent that can increase yield in the genetic background of cultivated rice (Brar and Singh 2011) w.r.t. panicle and plant height, suggesting it may have played a role in the domestication of rice. Studies of QTL or genes for yield and yield components being attributed to the wild donor parents, not only belongs to ancestral A-genome species, O. rufipogon or O. nivara, but also in the more distant tetraploid O. minuta with a BBCC genome (Brar and Singh 2011). Observations confirm that not only single genes and alleles are affecting yield traits but there are epistatic interactions and epigenetic interactions, as well as environmental factors affecting many of yield traits, resulting as transgressive variation.



3.1.3.2. Photosynthetic efficiency and yield improvement: Improving leaf photosynthetic efficiency (P<sub>max</sub>) to increase the crop yield is a quite extensively studied area, which has tremendous potential for yield improvement. Along with Pmax, other leaf features viz. leaf morphological and anatomical features including leaf area and orientation, organization of mesophyll and vasculature, strongly determine overall photosynthetic process and yield. Theoretically, it is possible to improve plant growth (and thus productivity and final biological or economic yield) either by increasing the amount of photosynthesis or by reducing 'unnecessary' respiratory costs or by allocating more C into appropriate sinks. Over the years, researchers had associated higher unit leaf photosynthesis with higher crop yield, but paradoxically, selection for higher or maximum net photosynthesis rate within a given species was often not associated with higher productivity (Austin1990). Notably, in the rice varieties released from IRRI between 1966 and 1980, there was a decline in  $P_{max}$ , stomatal conductance, leaf protein, chlorophyll and Rubisco content, whereas the values increased in the varieties released after 1980. It was suggested that the grain yield in IRRI varieties released prior to 1980 was correlated with harvest index, whereas, it was correlated with total plant biomass, in the varieties released after 1980 (Hubbart et al. 2007).

## 3.2. Indian works

3.2.1. Early Rice improvement: In India, early rice improvement was mainly dealing with improvement of popular local varieties through pure line selection. Several improved varieties were evolved, viz., T 141, T 1242, Latisail, Manoharsali, MTU 15, CO 25. Similarly, varieties suitable for specific biotic and abiotic stress situation were also developed. These varieties as evolved from landraces and farmers variety, hence mostly suitable for low management condition and had the ability to tolerate stress to some extent, but were not promising for yield enhancement. Establishment of Central Rice Research Institute (CRRI), Cuttak in 1946 by the Govt. of India, was a turning point in the history of rice research and provided a momentum to it. Inter-racial hybridization programme between japonicas and indicas during 1950-54 by The Food and Agriculture Organization of the United Nations had resulted a limited success. Only four varieties, viz., Malinja and Mashuri in Malaysia, ADT-27 in Tamil Nadu, India and Circna in Australia were released from more than 700 hybrid combinations (Parthasarathy1972). However, one variety, Mashuri was used extensively in breeding programmes as parent of present day mega varieties, viz., Swarna and Samba Mashuri. Since lodging was a major handicap for tall *indica* varieties, initiative was taken for improvement of weak stem to stiff straw genotypes with less lodging, with the help of short statured tropical japonicas, viz., Taichung 65, Tainan 3 and Waikyo Ku.

**3.2.2. Recent works at NRRI, Cuttack:** Yield improvement work was initiated following ideotype concept to break yield ceiling. In this context, New Generation Rice (NGR) has been conceptualized with an objective of modest grain yield of 10.0t/ha under farmers' field condition with a favorable management condition, notwithstanding the limitation of low light condition of eastern India. The plant type of this rice is basically contributed by following traits.



- Semi dwarf but with slightly raised height (Around 110cm).
- Strong culm to resist the moderate wind speed at maturity stage.
- Top three leaves should be erect with high specific leaf weight and v-shaped.
- Moderately high tillers (8-10 all effective).
- Moderately high grains (250-300).
- Moderately heavy panicle (5g or more).
- Field tolerance to major disease and pests
- Acceptable 1000 grain weight (21.0-24.0 g 1000 grain weight) and good quality parameters.
- Ability to continue higher photosynthesis even during Grain Filling Stage (7-25 DAF).
- Maturity duration of 130-145 days for irrigated and favorable shallow lowland.
- Yield potential of 10.0 t/ha or even more under low light condition of eastern zone.

The second generations NPTs developed at IRRI were collected in the segregating stage, and the further trait specific selection was exercised to establish fixed lines, i.e., NPT selections (NPTs). These NPTs performed exceptionally well, and even some of those showed the productivity of more than 10.0 t ha<sup>-1</sup> during dry season 2011(Dash et al. 2015). However, a super rice variety is still a need of the day, which should have productivity potential of at least 20% higher than the popular mega varieties or best check vis-à-vis resistance to abiotic and biotic stress along with acceptable grain quality. Moreover, it should have stable yield performance in multiple sites even in moderate low light stress. There is no such report of super rice in India till date. Hence, with an objective of development of indigenous super rice, the NPT lines along with standard popular indica check varieties and tropical japonica lines were selected for study, for identification of divergent gene pools in the backdrop of high yield under lowlight. The divergence analysis revealed that NPT selections had clustered differently and maintains sufficient diversity with respect to tropical japonicas, temperate japonicas, derivatives of indica/ temperate japonicas and even specific popular indica varieties. Therefore, NPTs could be potentially exploited for recombination breeding with these genotypes. Similarly, Garris et al. (2005) could detect five distinct groups, corresponding to *indica, aus*, aromatic, *temperate* japonica, and tropical japonica rice. Nuclear and chloroplast data supports a closer evolutionary relationship between the *indica* and the *aus* and among the *tropical japonica, temperate japonica*, and aromatic groups.

It was followed by study of combining ability analysis and some of the NPTs, viz., IR 73963-86-1-5-2-2, IR 72967-12-2-3 and IR 73907-753-2-3 were found to be excellent general combiners, although were not toppers in grain yield category. In this context, these were hybridized with a set of promising *tropical japonica*, *indica* and aromatic lines with potential yield and yield attributing traits. Out of 400 fixed lines few could



be found with traits matching to the NPT characters and increment in grain yield to the tune of 19-55% in comparison to popular indica check variety Swarna. Culture CR 3856-44-22-2-1-11 obtained from the cross IR 73963-86-1-5-2-2 and CR 2324-1 was found to be one of the top runner with yield potential of 11.2 tha-1 (10.8 tha-1 and 10.4 tha<sup>-1</sup> during 2016 and 2017, respectively in farmers field) (NRRI 2017). Coming to the traits attributing grain yield, it was found that the high grain yield was obtained due to heavy panicles (6-8 gpanicle<sup>-1</sup>), high grain number (250-300) with good quality (medium slender grains, 22.0g per 1000 grains), shy tillering (6-7), raised plant height (115-120cm), long semi erect top three leaves (length 39.0 cm, 44 and 46cm for 1st, 2nd and 3<sup>rd</sup> respectively; width 2.4 cm average for all three). Again to support heavy panicles, it has strong and thick culm (Fig.3 and 4). However, it is also associated with some bottlenecks which need improvement for further yield increment as well as stability. It needs reduction in height to maximum 110 cm and the culm strength has to be enhanced further to withstand the untimely heavy wind occurs during fag end of cropping season. The spikelet sterility also needs to be reduced from 20% to 10%. Moreover, it should be incorporated with resistance for BLB and few others disease and pests.



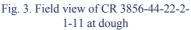




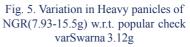
Fig. 4. Single plant of CR 3856-44-22-2-1-11

Similarly another variety Maudamani (CR Dhan 307) (Parentage: Dandi/Naveen / / Dandi) has been released for irrigated ecosystem during 2015 has shown promising

yield potential of 11.5 tha<sup>-1</sup> (7-11.5tha<sup>-1</sup>) under farmers field condition. It has also heavy panicles (6-8g), high grain number (250-300) with short bold grains (1000grain weight: 24.6g). It is suitable for irrigated ecology and endowed with characters viz., moderately strong culm, medium tillers (7-8), wide top leaves (2.2cm) and v-shaped stiff leaves and stay green character.

Many genotypes were with heavy panicles (7.93 g to 15.5g, Fig.5) were selected from different crosses of NGR.







However, all of them could not be translated into higher grain yield due to inferior population performance. This may be either due to non-uniform panicle type, or may be due to less tillers or high spikelet sterility. Again, these NGRs with heavy panicles were found to be more prone to biotic stresses. Therefore, trait specific supplementation along with incorporation of disease/pest resistance would make these NGRs more stable. Classical recombination breeding and markers assisted backcrossing for specific traits could be the options for augmentation of characters to make these high yielders stable.

Another focus at NRRI is given to develop NPT/Super rice varieties with increased photosynthesis and grain yield. The highest erecto-foliage leaf orientation coupled with highest photosynthetic rate (35.2 - 49.1 µmole  $CO_2 m^{-2} s^{-1}$ ), maximum photosynthetic quantum yield efficiency of PS II ( $F_v/F_m$  ratio of 0.770 - 0.808) with high performance index (2.21 - 3.84), high biomass (10-11 tha<sup>-1</sup>), high HI (0.52), high panicle number (340) and higher grain filling percentage (>85%) are key traits contributing for higher yield potential (6-8 tha<sup>-1</sup>) in some NPT lines. Higher LAI (5.0-6.3), high  $P_{max}$  (40-43 µmole  $CO_2 m^{-2} s^{-1}$ ), higher biomass (13-15 tha<sup>-1</sup>), high HI (0.42-0.50), higher panicle no (316-400), and higher translocation efficiency with high grain filling percentage (>80%) contributed high grain yield of more than 6.5 t ha<sup>-1</sup> with yield advantage of 0.5 - 1.0 t ha<sup>-1</sup> over the checks in NGR lines IR 73895-33- 1-3- 2, IR 73907-75- 3-2-3 and IR 73896-51-2-1- 3 (NRRI 2012). Proper physiological complementation with existing NGR would definitely help to attain new heights of productivity.

**3.2.3. Future generation rice:** Indian Institute of Rice Research, Hyderabad has conceptualized "future generation rice" for breaking yield ceiling. It started with screening of tropical *japonica* and selection of promising accessions as donors. Popular and highly adopted varieties (NDR 359, Swarna) were taken as recurrent parent and back crossed with these accessions. In BC,F, stage, the elite lines were intercrossed and generation advancement was done for necessary fixation. Initial selected lines or 1st generation plant types were having traits, viz., semi dwarf height, heavy panicles, *indica* type grains but with poor grain filling. It was also having undesirable feature, viz., early senescence and was susceptible to major disease and pests. However, after selected intermating within and between populations there was improvement in grain filling, stem thickness, panicle length and duration of senesces. Some of the genotypes were having ideal traits, viz., Plant height: 110-120cm, No. of panicles per plant: 6-10, high grain number, strong/thick culm, test weight: 20-24g, late senescence, shy tillers but with very less unproductive tillers with a duration of 120-145 days. These are having high biomass with 48-50% HI and very high yield potential. These lines are under national multi location testing. Similarly, some *O.rufipogon* derived lines have shown improvement in biomass as well as sink size and could be used as prospective parents in improving yield of present day varieties and parental lines.



# 4. AGRONOMIC MANAGEMENT FOR NEXT GENERATION RICE

Potential productivity of any variety is accomplished not only by genetic potential, but also by optimum agronomic management. Thus, it is imperative to understand yield responses of NPT/super rice/ NGR to various agronomic practices. As NGR has different morpho-physiological attributes, it necessitates new management practices. Among the practices, nutrient management, crop establishment, water management and pest management are the key factors in realizing the potential yields. Many experiments have been conducted to analyze the nutrient accumulations in different rice varieties and the variation is enormous across soil, climate, location and management. However, limited literature is available on the management of super rice. Reports suggest that establishment methods have differential effects on nutrient uptake, crop growth, weed occurrence, and subsequently crop yield (Singh et al. 2006).

At National Rice Research Institute, superior grain yield was reported by application of 120 kg Nha<sup>-1</sup> in NPT cultures than 80kgha<sup>-1</sup>. Higher N dose of 160 kgha<sup>-1</sup> was not found to have any positive effect on the yield. As NPT were having some sort of shy tillering, closer spacing (15x15 cm) was found to have high yield than normal/higher one (20x20cm or 20x15cm) (NRRI 2013-14). Similarly, multi location trials conducted under AICRIP recorded higher average grain yield of NPT genotypes in closer spacing (15x15 cm) w.r.t. normal (20x20cm), whereas, other check varieties experienced yield reduction in closer spacing (IIRR 2017).

However, further research is needed to have a comprehensive and more quantifiable package of practices for NGR.

## 5. KNOWLEDGE GAPS AND SOLUTION

In future, production of rice also needs to be increased from lesser land area due to population explosion and shrinkage of resources. As there is hardly any scope of horizontal expansion, the vertical expansion is the only way out. For this purpose, there is continuous effort towards increasing grain yield by means of higher plant population per unit area, higher per plant yield though higher grain number per panicle, higher spikelet fertility and better grain weight. This will definitely increase the weight of upper part of the plant and thereby increasing the chance of lodging in *indica* rice. The problem is further coupled by increasing climatic vagaries like erratic rainfall, increase in extreme weather events and uncharacteristic wind flow especially in eastern and southern coasts of the country. Without improving the actual strength of culm, it will not be possible to break the yield ceiling of rice, as our target will be to produce the genotype of high biomass coupled with higher harvest index. Reduction of height to semi-dwarf stature is definitely the option, but relying solely on it might be having some limitations, which necessitates use of other important traits to reinforce it. Fortunately, several such useful genes as well as precisely mapped QTLs are now



available for the breeder (Table 1), which will definitely help in designed breeding of NGRs. However, finding the suitable combinations of these genes/QTLs are highly essential. Yield being a highly complex trait is subjected to differentiation in component traits. However, the major challenge is avoiding the negative trade-off among those component traits. Hirano et al. (2017) suggested the use of combination of mild alleles of the genes for these negative trade-off combination traits rather than use of strong alleles, which often accompany undesirable side effects on other yield components (e.g., strong alleles for *APO1* and *OsTB1* although increases grain number per panicle and culm strength of plants, significantly reduces the tiller number). However, while pyramiding for same trait, combining even mild alleles with similar mode of function may again show detrimental effect on phenotype. With increasing knowledge gained on mode of molecular function of the genes, it is easier to develop plants with ideal traits of NGR, which can break the long pending yield ceiling of *indica* rice.

## 6. CONCLUSION

The stagnation of yield of existing high yielding rice varieties call for breaking the yield barrier for meeting food demand of ever increasing population. Grain yield is a complex character, where many genes and QTLs have intricate interaction with several physiological and bio-chemical processes. Therefore, a breakthrough in yield potential requires a comprehensive research and improvement of all the aspects that affect grain yield and the factors affecting its production in view of changing climatic scenario. Selection for morphological characters with physiological implication may be the first choice for crop improvement. However, it should be supplemented for molecular breeding for higher agronomical and physiological efficiency. Improvement of harvest index by researchers is in focus in many grain crops including rice, but as it is approaching a ceiling, increasing its potential has to involve an increase in biomass which has to be achieved through increasing photosynthesis. Empirical breeding for population improvement has resulted in a high productivity of rice for last 30 years in tropics. Modification of plant type and utilization of heterosis are two primary strategies now being used to increase the yield potential of irrigated lowland in the tropics. Intra-varietal cross between indica types has only limited scope of yield improvement. However, intersub-specific hybridization between tropical japonica and indica has a great potentiality for increment in production potential. There are several unexploited sources including wild rice which has already shown promise and would play a great role in future. Prospective rice improvement has to address the issues of identification of physiological basis of morphological traits, their GxE interaction for controlling grain yield. Genomic assisted breeding with introgression of QTLs/genes for quantitative traits (for higher grain yield) has immense potentiality for supplementations of characters for attaining new heights in grain yield. However, this has to be environment specific and clear-cut management options need to be developed for expressing its full potential for breaking yield ceiling.



#### References

- Aggarwal PK, Kropff MJ, Teng PS and Khush GS (1996) The challenge of integrating systems approaches in plant breeding: opportunities, accomplishments and limitations. In: EdsKropff MJ, Teng PS, Aggarwal PK, Bouman BAM, Bouman J & Van laar HH. Application of systems approaches at the field level. Kluwer Acadamic publishers, Dordrecht, the Neitherlands, pp.1-24.
- Austin RB (1990) Prospects for genetically increasing the photosynthetic capacity of crops. No. 91-042105. CIMMYT.
- Bose LK (2005) Broadening gene pool of rice for resistance to biotic stresses through wide hybridization. Iranian Journalof Biotechnology 3(3): 140-143.
- Brar D and Singh K (2011) Oryza. In: Kole C, editor. Wild crop relatives: Genomic and breeding resources: Cereals. Dordrecht London, New York: Springer Heidelberg, pp. 321-36.
- Chandler RF (1982) An Adventure in Applied Science: A History of the International Rice Research Institute. International Rice Research Institute, Los Baños, Philippines, 233 pp.
- Chung GS and Heu MH (1980) Status of *japonica-indica* hybridization in Korea. In: Innovative approaches to rice breeding. Selected papers from the 1979 International Rice Research Conference pp. 135-152.
- Dash SK, Meher J, Behera L, Anandan A, Azharudheen TPM, Barik M and Singh ON (2015) Genetic diversity of New Plant Type rice selections with reference to indica, tropical japonicas, temperate japonicas and irrigated cultures. Oryza 52(4):266-274.
- Dingkuhn M, Penning de Vries FWT, De Datta SK and Van Laar HH (1991) Concepts for a new plant type for direct seeded flooded tropical rice. In: Direct seeded flooded rice in the tropics. International Rice Research Institute, Los Ban<sup>o</sup>s,Philippines, pp. 17–38.
- Donald CM (1968) The breeding of crop ideotypes. Euphytica 17: 385-403.
- Hirano K, Ordonio RL and Matsuoka M (2017) Engineering the lodging resistance mechanism of post-Green Revolution rice to meet future demands. Proceedings of the Japan Academy, Series B 93(4):220-233.
- Huang Y (2001) Rice ideotype breeding of Guangdong Academy of AgriculturalSciences in retrospect.Guangdong AgriculturalSciences 3:2–6
- Hubbart S, Peng S, Horton P, Chen Y and Murchie EH (2007) Trends in leaf photosynthesis in historical rice varieties developed in the Philippines since 1966. Journal of Experimental Botany 58(12):3429-3438.
- IIRR (2017) Progress Report 2016, Vol.1, Varietal Improvement. All India Coordinated Rice Improvement Project. ICAR-Indian Institute of Rice Research, Rajendranagar, Hyderabad – 500 030, T.S, India.
- Khush GS, Coffman WR and Beachell HM (2001) The history of rice breeding: IRRI's contribution. In: Rice research and production in the 21st century: Symposium honoring Robert F. Chandler Jr. International Rice Research Institute, Manila, Philippines pp. 117-135.
- Li and Ali (2016) http://books.irri.org/GSR-flyer.pdf. Accessed on 08.01.2017.
- Lu G , Coneva V, Casaretto JA, Ying S, Mahmood K, Liu F, Nambara E, Mei Bi Y, Rothstein S (2015) OsPIN5b modulates rice (Oriza sativa) plant architecture and yield by changing auxin homeostasis, transport and distribution. Plant J. 83: 913–925.
- Liu, L, Tong H, Xiao Y, Che R, Xu F, Hu B, Liang C, Chu J, Li J, Chu C.(2015) Activation of Big Grain1 significantly improves grain size by regulating auxin transport in rice. PNAS 112, 11102–11107 Los Baños, Philippines, pp. 135–152.



- NRRI (2017) Annual Report 2016-17. Developmet of super rice for different ecologies. ICAR-NRRI, Cuttack, pp. 47.
- NRRI (2012) Annual Report, 2011-12. Physiological characterization of NPT lines. ICAR-NRRI, Cuttack, pp. 35.
- NRRI (2014) Annual Report, 2013-14. Standardization of agronomy for super rice. Genetic Improvement of Rice. ICAR-NRRI, Cuttack, pp. 35.

Parthasarathy N (1972) Rice breeding in tropical Asia up to 1960. Rice Bred. 5-29.

- Paul M, Nuccio M and Basu SB (2018) Are GM Crops for Yield and Resilience Possible?.Trends in Plant Science 23(1):10-16.
- Peng S, Khush GS, Virk P, Tang Q and Zou Y (2008) Progress in ideotype breeding to increase rice yield
- Reves LC (2009) Making rice less thirsty. Rice Today July-September, pp. 12-15.
- Singh BD (2002) Plant breeding.Kalyani Publishers. Rajinder Nagar, Ludhiana-141008.
- Singh S, Bhushan L, Ladha JK, Gupta RK, Rao AN andSivaprasad B (2006) Weed management in dry-seeded rice (*Oryza sativa*) cultivated in the furrow-irrigated raised-bed planting system. Crop Protection 25:487-495.
- Swamy BM and Sarla N (2008) Yield enhancing QTLs from wild species of rice. Biotechnology Advances 26:106–120.
- Tanksley SD and McCouch SR (1997). Seed banks and molecular maps: unlocking genetic potential from the wild. Science 277(5329):1063-1066.
- Tian F, Li DJ, Fu Q, Zhu ZF, Fu YC, Wang XK and Sun CQ (2006) Construction of introgression lines carrying wild rice (*Oryza rufipogon*Griff.) segments in cultivated rice (*Oryza sativa* L.) background and characterization of introgressed segments associated with yield-related traits. Theoretical andApplied Genetics 112:570–580.
- Tsunoda S (1962). A developmental analysis of yielding ability in varieties of field crops. IV. Quantitative and spatial development of the stem-system. Japanese Journalof Breeding 12:49–55.
- Wang CL, Zhang YD, Zhu Z, Zhao L, Chen T and Lin J (2008) Characteristics and cultivationtechniques for the new rice variety 'Nanjing 46' with good eating quality, high yield and stripevirus resistance. Jiangsu Agricultural Sciences 2:91–92.
- Wang S, Wu K, Yuan Q, Liu X, Liu Z, Lin X, Zeng R, Zhu H, Dong G, Qian Q and Zhang G (2012) Control of grain size, shape and quality by OsSPL16 in rice. Nature Genetics 44:950.
- Wang Y, Kuroda E, Hirano M and Murata T (1997). Analysis of high yielding mechanism of rice varieties belonging to different plant types: I. Comparison of growth and yield characteristics and dry matter production. Japanese Journal of Crop Science 66(2):293-299.
- Xie X, Song HM, Jin F, Ahn SN, Suh JP and Hwang HG (2008) Fine mapping of a grain weight quantitative trait locus on rice chromosome 8 using near-isogenic lines derived from a cross between *Oryza sativa* and *Oryzarufipogon*. Theoretical and Applied Genetics 113:885– 894.
- Yang S, Zhang L, Chen W, Xu Z and Wang J (1996) Theories and methods of rice breeding for maximum yield. Zuowuxuebao 22(3):295–304.
- Yang T, Zeng R, Zhu H, Chen L, Zhang Z, Ding X, Li W, Zhang G (2010) Effect of grain length gene GS3 in pyramiding breeding of rice. Mol Plant Breed 8:59–66.



- Yao S, Chen T, Zhang YD, Zhu Z, Zhao QY, Zhou LH, Zhao L and Wang CL (2010). Pyramiding of translucent endosperm mutant gene Wx-mq and rice stripe disease resistance gene Stvbi by marker-assisted selection in rice (*Oryza sativa*). Chinese Journal of Rice Science 18:102–109.
- Ying JZ, Chen YY and Zhang HW (2014) Functional characterization of genes/QTLs for increasing rice yield potential. In: Rice-Germplasm, Genetics and Improvement. InTech.
- Yoshida S (1972). Physiological aspects of grain yield. Annual Review of Plant Physiology 23:437–464.
- Zhang YC, Yu Y, Wang CY, Li ZY, Liu Q, Xu J, Liao JY, Wang XJ, Qu LH, Chen F and Xin P (2013) Overexpression of microRNA OsmiR397 improves rice yield by increasing grain size and promoting panicle branching. Nature Biotechnology 31(9):848-852.\*



# Biotechnology for Rice Improvement: Achievements and Challenges

S Samantaray, N Umakanta, JL Katara, C Parameswaran, RL Verma, H Subudhi, A Kumar, Devanna BN and S Roy

## SUMMARY

Biotechnological strategies such as in-vitro culture, transgenics and genome editing (CRISPR/Cas9) provide immense opportunities for rice improvement. These tools have been effectively utilized for development of new varieties, mapping of QTLs, characterization and functional validation of genes, development of novel variants etc. However, indica rice requires special emphasis for improving callusing potential of anthers, albino-free green shoots regeneration in androgenesis, increasing transformation efficiency and optimization of delivery methods for CRISPR/Cas9 vectors. We could increase the callusing ability of anthers in indica rice up to 30% using combinations of media components and achieved 100% green shoot regeneration in androgenesis. The complex traits such as heat tolerance in rice requires multipronged approach through fine mapping, characterization of fixed alleles of heat stress responsive genes to understand the tolerance mechanism and effective introgression the genes through molecular approaches. Gain/loss -of-function alleles generated through CRISPR/Cas9 in farmers preferred varieties could modify the trait of interest and also maintain the aesthetic value of the variety. Thus, addressing critical issues such as callusing potential in androgenesis, higher transformation and genome editing efficiency in indica rice could assist in development of improved rice varieties for yield, stress tolerance and quality under this demanding climate change driven agriculture.

## **1. INTRODUCTION**

With the expanding growth of world population and gradually deteriorating environment, food security has become a major challenge around the world especially in rice growing countries. Increasing rice yield has become the most important goal of rice production on less land with limited resources. Hence, there is a sustained need focus on development of high yielding rice varieties with tolerance to biotic and abiotic stresses (Hasan et al. 2015). Though rice breeding efforts over the past six decades have contributed tremendously to the genetic improvement of rice in terms of yield and quality, traditional approaches suffer from several limitations to increase crop yield and productivity indefinitely. Conventional rice breeding is a slow process, typically requires 8-10 years from initiation to varietal release which also mostly depends on environmental conditions. Alternatively, biotechnology in recent years, has provided a powerful means to supplement traditional methods through the use of



molecular genetics in cloning and sequencing of genes leading to the analysis of the genome structure, evolution and expression.

The first decade of this century brought in revolution of DNA sequencing wherein the whole genome sequence of Arabidopsis, rice, sorghum, Medicago etc. were accomplished and made available in public domain. The genome sequences assisted in identification of genes, pathways, and understanding of mechanism of several developmental and stress response in plants. Additionally, identification of genome wide molecular markers helped in mapping of several important traits in plants. This development was quite useful in production of stress tolerant, high yielding varieties through marker assisted introgression. Similarly, genetic transformation shows its significance in development of agronomically important traits such as herbicide tolerance, shelf life, insect and disease resistance etc. in a number of crops. Besides, doubled haploid (DH) approach has significantly reduced the time required for development of mapping population and hastens the mapping of tolerance/resistance genes. Another latest technology known as CRISPR/Cas9 system could revolutionize the plant biology by editing major genes for crop improvement. Thus, DH breeding, transgenic approaches and CRISPR/Cas9 provide varied solutions to the need of biotechnologist in their quest for rice improvement which were highlighted in this chapter. Since the whole work on advances of these technologies particularly DHs and transgenics cannot be covered here, it was considered summarizing some important findings useful for rice improvement along with our own research dealings with androgenesis in production of DHs and transgenics for high temperature tolerance.

## 2. DOUBLED HAPLOID IN RICE IMPROVEMENT

To meet the challenge of food security for the increasing population amid diminishing resources like cultivable land and irrigation water along with climate change associated unpredictable and unseasonal weather patterns, development of high yielding rice varieties is need of the hour. Hybrid rice is considered as a best option to break the yield barrier with significant yield advantages over the conventional cultivars. Though hybrid rice can out-yield conventional cultivars by 30-40% in production fields, it has not gained its popularity among the Indian farmers due to its complicated seed production system, non-replacement of seed every season, higher seed cost, less preferred qualities and vulnerable to abiotic and biotic stresses. Therefore, it is required to find out an alternative way to exploit the hybrid potential by fixing heterosis along with the associated problems, for which DHs technology was found efficient in rapid fixation of favourable alleles for yield and related traits. The DH breeding technique shortens the time required for breeding a new variety from the usual  $\sim 8$  years to  $\sim 5$  years (Fig. 1), thus saving on time, labour and financial resources. Conversely, DH technique could be more appropriate for developing new varieties from photosensitive rice genotypes. Most of these advances were achieved



in japonica cultivars which are more amenable to anther culture than indica rice. Significantly, a number of varieties and improved parental lines have been developed through androgenic approaches, but it is only restricted to japonica rice cultivars. However, the use of anther culture as a routine technique for breeding is extremely limited in indica rice due to poor induction of androgenic calli and subsequent plant regeneration. To alleviate the problems associated with the indica hybrid rice cultivation, anther culture could be employed to develop DH recombinants that performed almost nearer to hybrids (Naik et al. 2016).

# 3. TRANSGENIC APPROACH IN RICE

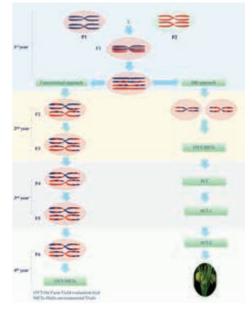


Fig.1. Doubled haploid vs. Conventional breeding: Duration for varietal release.

Climate change could drastically reduce the productivity of crops in developing countries like India for which finding a sustainable solution is necessarily required to address such problem. Biotechnological interventions have been successfully utilized in stacking of several genes within a short span of time to cope with the prevailing situations. Since the simple traits were controlled by qualitative genes, most of the quantitative traits governed by several genes such as drought, salinity, heat were dependent on transgenics. Even though, the transgenic approaches are highly successful in developing tolerance, validation of transgenic plants in most cases has been reported only in control conditions such as phytotron or green house facility. The evaluation of transgenic in field condition is very much essential to understand the tolerance at field level. Thus, the constraints for using biotechnological tools for crop improvement of complex traits are significant. Though the transgenic approach plays a significant role in crop improvement, there are limitations for the successful gene transfer in several crops.

Genetic engineering in rice has been utilized to transfer several genes for important traits such as yield, quality and tolerance to biotic and abiotic stress tolerance. *Cry1Ac* gene tolerant to rice pests was transformed for enhancing insect tolerance in rice (Lee et al. 2016). Moreover, ferritin was used to increase the iron content in rice endosperm through genetic engineering (Masuda et al. 2013). The rice vacuolar Na<sup>+</sup>/H<sup>+</sup> antiporter gene were used to enhance the salinity tolerance in rice (Reddy et al. 2017). Over expression of DREB genes has been shown to increase the cold tolerance in rice (Cruz et al. 2013). Thus, transgenic approaches in rice have contributed significantly in development of biotic/abiotic stress tolerance and quality improvement of rice.



# 4. CRISPR/CAS9 TECHNOLOGY IN RICE GENOME EDITING

Due to gradual decline in genetic variation, the cultivated crops become more vulnerable towards abiotic and biotic stresses. Climate change and human activities are also contributing to this factor. Besides, natural gene pool available in wild ancestors and landraces are useful to understand important biological mechanisms. However, useful genetic resources from the wild genetic pool have been transferred in cultivated rice with limited achievements to improve the genetic constituent of cultivated crops. Conventionally, induced mutation or target mutagenesis has been adopted to create variation in the cultivated gene pool which is beneficial for genetic improvement of cultivated crops. In the past decades, induced mutation technology such as physical, chemical or biological (T-DNA or transposon insertion) mutagenesis have been widely used to identify novel mutants in model plants like Arabidopsis and rice. However, such random mutagenesis produces many undesirable mutations and genome rearrangements and screening of large scale mutants remains tedious and costly (McCallum et al. 2000). Therefore, there is urgent requirement for a revolutionary robust technology of targeted mutagenesis for genetic improvement of traits in crops. Genome editing is a new technology widely used in the studies of functional genomics, reverse genetics, genome engineering and targeted transgene integration by allowing of addition, deletion or alteration of genetic material at particular locations in the genome in an efficient and precise manner. In general, it involves the introduction of targeted DNA double-strand breaks (DSBs) using an engineered nuclease, which can induce site-specific changes in the genomes of cellular organisms through a sequence-specific DNA-binding domain and a non-specific DNA cleavage domain thereby generates desired insertions, deletions or alterations.

## 5. STATUS OF RESEARCH

The past and current status of research highlights the usefulness of DHs, transgenics and CRISPR/Cas9 technology in rice improvement.

#### 5.1. Manipulation of factors in success of anther culture

The discovery of haploids in plants led to the use of DH technology in plant breeding. Though there are different methods to generate haploids and then obtain DHs by chromosome doubling, *in vitro* methods were found to be most suitable for production of DHs. There are two *in vitro* methods i.e. gynogenesis and androgenesis available for DH production from which androgenesis shows its effectiveness and applicability in production of haploids and DHs in numerous cereals including rice. These systems allow completely homozygous lines to be developed from heterozygous parents in a single generation.

The first naturally occurring haploids were reported by Blakeslee et al. (1922) in jimson weed (*Datura stramonium*) and thereafter natural haploids were documented in several other species. However, the relevance of DHs came into attention only



when Guha and Maheshwari (1964, 1966) reported a breakthrough in the production of haploids from anther culture of Datura (Datura innoxia). Further, their research revolutionized the use of DH technology in plant breeding worldwide. Subsequently, this haploid discovery by anther culture provided several opportunities for application of this technique in crop improvement programs. In rice, first report on production of haploids through anther culture was reported by Niizeki and Oono (1968). Thereafter, doubled haploidy approach coupled with conventional breeding led to the development of a number of rice varieties for pest and disease resistance, high yield, and quality grains. In China, several varieties of rice viz. Xin-Xin, Hua-Hau-Zao, Zhong-Hua-8, Zhong-Hua-9, Zhong-Hua-10, Zhong-Hua-11, Hua Yu-1, Hua Yu-2, Tanfong1, Nonhau5, Nonhau11, Aya and ZheKeng 66 possessing high yield, superior quality, tolerance to abiotic stress such as cold, early maturity, resistance to disease have been released through the use of DHs. Further, in Japan, several successful rice varieties developed through anther culture techniques are Joiku N. 394, Hirohikari, Hirohonami AC No.1 and Kibinohana which are tolerant to cold and are good in taste. Similarly, two rice varieties (Patei and Moccoi) and one rice variety (Dama) were released in Argentina and Hungary, respectively along with two rice varieties in Republic of Korea also employing DH approach. In India, Satyakrishna (CR Dhan 10) and Phalguni (CR Dhan 801) are the first released indica rice varieties from DH lines (CRRI Annual Report, 2008-09, 2010-11; www.crri.nic.in). Besides, a rice variety "Parag 401" has also been bred through DH breeding. Furthermore, anther culture could facilitate other biotechnological approaches such as gene transformation and identification of QTLs.

Despite all the advantages DH technology offers, it has not been put to use in the country to that extent to take maximum advantage. This is primarily due to lack of expertise and variable response of different genotypes under in vitro culture. Though androgenic response to japonica type has led to release of many varieties, the potential of the anther culture technique for indica rice breeding is not fully exploited in spite of releasing a salt tolerant indica variety through anther culture (Senadhira et al. 2002). Early anther necrosis, poor callus proliferation and albino-1 plant regeneration are some of the problems encountered in case of indica rice at the time of androgenesis which require vast improvement. The genetic diversity is also a determining factor in the success of anther culture.

Physiology of the donor plant is an important contributory factor for the success of rice anther culture. Anthers of panicles collected from field grown plants have been decidedly better in their anther culture response compared to anthers collected from pot plants placed in the green house or near the field (Veeraraghavan 2007). Usually, the distance between the collar of the flag leaf and ligule of the penultimate leaf of the tiller serves as a reliable guide to anther maturity. Secondly, microspore stage is considered as an important factor for androgenic response. An easily observable morphological trait of the plant that shows good correlation with the pollen development stage is used as a guide to identify the required stage of microspore (Nurhasanah et al. 2015). The most suitable stage of microspore development has



been described as the late uni-nucleate to early bi-nucleate stage as well as early to mid uni-nucleate stage.

A wide range of chemical and physical factors influences the androgenesis *in vitro*. The most widely used pre-treatment for androgenesis is the low temperature shock for specific duration. Mishra et al. (2013) assessed the influence of cold pretreatment at 10°C for 7-9 days on the anther culture response of Rajalaxmi (CRHR 5) and Ajay (CRHR 7) which showed a positive influence on the callus induction frequency irrespective of the media and PGRs employed; prolonged treatment over the optimum proved to be inhibitory for androgenesis. However, cold treatment (10 °C) for 8 days was found to be effective for callus induction and green plant regeneration in a popular indica rice hybrid, BS6444G (Naik et al. 2017). Besides, two days pre-incubation period at 10 °C was quite interesting for the success of androgenesis in a long duration indica rice hybrid (Rout et al. 2016).

The most commonly used basal media for anther culture are N6, MS, B5 and Potato-2 medium. Subsequently, several media (MSN, SK1, He2 and RZ) were developed from the N6 media modifying the nitrogen levels and sources, carbon level and sources, changes in vitamins and their concentrations which were found to be encouraging for anther response in rice. N6 media was found to induce maximum callusing in Taraoi Basmati (Grewal et al. 2006). Minj et al. (2016) could find out the best media for androgenesis in generation of DHs from  $F_1$ s of two inter-varietal crosses in terms of callusing (N6) and shoot regeneration ( $\frac{1}{2}$  MS) after trial with 16 different media. However, two basal media such as N6 and MS media were found to be effective for callusing and green shoot regeneration, respectively in indica rice hybrids (Rout et al. 2016; Naik et al. 2017).

Considering the importance of plant growth regulators in tissue culture, the effects of different PGRS were investigated for androgenesis. Even though, 2,4-D has proven to be a potent auxin for callus induction from cultured anthers, however, medium with lower 2,4-D levels was found to be more effective for the regeneration ability of callus induced in indica rice as compared to higher 2,4-D levels in *japonica* rice (Naik et al. 2017). Media supplemented with NAA (0.5 mg/l), BAP (1.0 mg/l) and Kn (1.0 mg/l) adequately supported green plant regeneration from sub-cultured callus (Rout et al. 2016). The type and the concentration of auxins seem to determine the pathway of microspore development with 2, 4-D inducing callus formation and IAA and NAA promoting direct embryogenesis (Ball et al. 1993). 2,4-D was effective for callus response while the combinations of NAA, Kn, BAP showed shoot regeneration in generation of DHs from F<sub>1</sub>s of two inter-varietal crosses (Minj et al. 2016).

The nitrogen composition supplied in the form of nitrate and/or ammonium ions in culture media plays a significant role for androgenesis. The ratio of nitrate (NO<sub>3</sub><sup>-</sup>) : ammonium (NH<sub>4</sub><sup>+</sup>) has been observed to be an important determinant for the success of anther culture as well as for the in vitro induction of embryogenic callus in indica rice (Grimes and Hodges 1990). Ivanova and Van Staden (2009) investigated the elimination of total nitrogen in media resulted in limited ability of proliferation and shoot growth but higher ability was observed in media containing NO<sub>3</sub><sup>-</sup> as the only



sole of nitrogen source and replacing the  $NH_4^+$  to  $NO_3^-$  decreased the rate of hyperhydricity. Herath et al. (2007) proved that frequency of callus induction was improved by modification of three different media (N6, B5 and Miller) with one half the level of  $NH_4^+$  and double the level of  $KNO_3$  nitrogen.

A carbohydrate source is essential for androgenesis because of their osmotic and nutritional effects. The superiority of maltose over sucrose as the carbon source in rice anther culture for callusing was aptly demonstrated by Naik et al. (2017). Replacing sucrose (146 mM) with maltose (146 mM) in the callus induction medium had a significant positive effect on anther response in both indica and japonica types with a greater effect on indica rice. With respect to the effect of light quality on anther culture, the embryogenic induction of microspores is inhibited by high-intensity white light whereas darkness or low-intensity white light are found encouraging (Bjornstad et al. 1989). The incubation of anthers continuously in the dark has, on occasion, been found to be essential.

Most of the *in vitro* morphogenic responses are genotype-dependent. In general, indica cultivars of rice exhibit poor androgenic response as compared to the japonica ones. Even among the indica cultivars, a considerable variation for pollen callusing and plant regeneration has been observed (Rout et al. 2016). Highest callus responsive cultivars often show the best regeneration frequency and also the best responsive genotypes to callusing exhibit low regeneration ability. Therefore, selection of a single step either callus improvement trait or shoot regeneration trait alone may not help in establish an effective androgenic method. It is rather important to identify genotypes carrying the two traits for overall improvement in anther culture efficiency.

#### 5.2. Transgenics for high temperature stress

Heat stress in crops is considered as one of the major threat to crop production by Intergovernmental Panel on Climate Change (IPCC) (Teixeira et al. 2013). Climate modeling analysis has predicted that 16% of rice growing areas will be subjected to five days of heat stress during reproductive stage (Jagadish et al. 2015). Heat stress in rice affects the spikelet sterility during anthesis and affects the grain quality during the grain filling stages. The spikelet fertility was reduced by 7% for every one degree increase above 30 °C in one of the famous rice variety IR64 (Jagadish et al. 2007). Rice is sensitive to heat stress during gametogenesis and flowering stages of the crop growth. The cardinal temperature for heat stress in rice is >35 °C (Prasad et al. 2006). In China, post heading heat stress has reduced rice yield by 1.5 to 9.7% during last three decades (Shi et al. 2015). High temperature (>36 °C) causes significant increase in spikelet sterility and also affects the grain quality in rice. There were 8 QTLs identified on different chromosome for heat stress tolerance using Nagina22 (N22) as donor parent (Ishimaru et al. 2016). The phenotypic variance of the identified QTLs varied from 11-25% for spikelet fertility during heat stress in rice. A high resolution phenotyping mapping of QTL using RIL population has identified novel QTL with effect up to 22% and also identified candidate genes within the QTL region (Shanmugavadivel et al. 2017).



### 5.3. Genome editing

Genome editing is a new technology widely used in the studies of functional genomics, reverse genetics, genome engineering and targeted transgene integration by allowing of addition, deletion or alteration of genetic material at specific locations in the genome in an efficient and precise manner. In general, it involves the introduction of targeted DNA double-strand breaks (DSBs) using an engineered nuclease, which can induce site-specific changes in the genomes of cellular organisms through a sequence-specific DNA-binding domain and a non-specific DNA cleavage domain thereby generates desired insertions, deletions or alterations. Different genome modifications/editing can be achieved depending on the two repair pathways: NHEJ; non-homologous end joining and HR; homologous recombination. Insertion and deletion are the common phenomena in NHEJ pathway which causes target gene knockout/disruption or the production of truncated proteins whereas HR normally relies on recombination with homologous sequences in an undamaged chromatid leads to the introduction of precise alterations to the genome, which are specified by the template (Chandrasegaran and Carroll 2016). In addition, genome editing technologies allow genome modification without the introduction of foreign DNA which would be helpful to edited crops that could be classified as non-GMO. Nucleasemediated editing of plants and agricultural animals may greatly decrease the time required to generate new varieties of both species relative to traditional breeding strategies.

#### 5.4. CRISPR/Cas9 in rice genetic improvement

CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR associated protein-9 nuclease) is a powerful and revolutionary technology for targeted mutagenesis in molecular biology research and genetic improvement of traits in diverse organisms including important crops (Ma et al. 2016). Unlike the protein-guided DNA cleavage of ZFNs and TALENs, CRISPR/Cas9 depends on small RNA for sequencespecific cleavage in genome sequence. The system is derived from bacterial innate immune system, the type II CRISPR/Cas system of Streptococcus pyogenes (Jinek et al. 2012). In bacteria, single nuclease Cas9 process the foreign sequences into small segments by cleavage and introduce them into the regularly interspaced palindromic repeats called CRISPR array and serve as templates for CRISPR RNA (crRNA). This crRNA then hybridizes with a trans-activating RNA (tracrRNA) and form a dual complex and guide Cas9 protein to detect and cleave the foreign DNA. In the present system, the dual complex of crRNA:tracrRNA, is modified into a single RNA chimera; this is called sgRNA (single guideRNA) which recognizes 20 or 24 nt sequences matching to target sites in the upstream of protospacer adjacent motif (PAM) (Jinek et al. 2012). This CRISPR/Cas9 construct targets specific DNA sequence with 20-24 nucleotides which is unique in most genomes, cleave and repair thereby inducing deletion or insertion mutation. It is particularly useful for those traits controlled by negative regulatory genes which can be improved simply by knockout or weakening of the gene expression. CRISPR/Cas9 system has been successfully used as an efficient tool for genome editing in a variety of crops (Ma et al. 2016). Identification of potential



known genes for yield improvement along with tolerance/resistant toward abiotic and biotic stress is pre-requisites for development of superior rice lines. The CRISPR/ Cas9 system enables precise targeting and cleavage causing random mutagenesis at the specific predetermined sequence/locus within a large genome. CRISPR/Cas9 technology has already shown its potentiality to rapidly and precisely edit specific plant genes of interest to achieve the desired outcomes.

The most common application of the targeted editing system in genetic improvement is to knock out completely the functions of target genes, usually by editing site(s) in the coding sequences (CDS) to produce null-allele mutants. The CRISPR/Cas9 based genome editing system has many applications for functional studies of plant genes. It has also provided a robust tool for genetic improvement of important traits such as yield, plant architecture, abiotic and biotic stress etc. For instance, Xie et al. (2017a; 2017b) adopted CRISPR/Cas9 system to edit SaF+ or SaM+ and OgTPR1 genes at the hybrid sterility loci, Sa and SI in rice for overcoming hybrid sterility in inter-subspecific and inter-specific hybrid rice breeding. This technology has been further utilized in studying the hybrid male sterility locus Sc in rice by reducing the tandem-repeated gene copy number in indica rice allele Sc-I to improve male fertility in japonica-indica hybrids (Shen et al. 2017). Shimatani et al. (2017) recently developed a fusion of CRISPR/Cas9 and activation-induced cytidine deaminase (Target-AID) system for point mutagenesis and successfully demonstrated editing in acetolactate synthase (ALS) enzyme that confers herbicide resistance in rice. Zhang et al. (2017) used CRISPR/Cas9 technology to validate the molecular function of OsFIGNL1 responsible for the male sterility in rice. In another study, knocking-out of Broad-Spectrum Resistance 1 (BSR1) using CRISPR/Cas9 system which encodes arice receptor-like cytoplasmic kinase showed highly susceptible to fungal pathogens (Kanda et al. 2017). Qiu et al. (2017) also cloned and characterized the gene heat-sensitive albinol (hsal) responsible for chloroplast development under heat stress in rice and deletion mutation in this gene induced by CRISPR/Cas9 system which was found to be heat sensitive. Nieves-Cordones et al. (2017) observed that inactivation of the (cesium) Cs+-permeable K+ transporter OsHAK1 with the CRISPR/ Cas9 system dramatically reduced Cs+ uptake in rice. Sun et al. (2017) demonstrated the feasibility of creating high-amylose rice through CRISPR/Cas9-mediated editing of SBEIIb gene.

#### 5.5. Doubled haploids production and application

National Rice Research Institute (NRRI), Cuttack initiated the work in 1997 on DH technique to overcome the constraints associated with the indica rice hybrids: 1) expensive seed depriving the Indian marginal farmers to utilize the seed year after year 2) unpredictable environmental condition and a synchronised flowering. Considerable progress of NRRI has been made as evidenced by release of two DHs as new varieties named Satyakrishna and Phalguniin in 2008 and 2010, respectively. In the year 2013, another attempt was made to standardize androgenic protocols in two more indica rice hybrids i.e., CRHR32 (an elite long duration rice hybrid developed at NRRI, Cuttack) and BS6444G (a popular rice hybrid, Bayer Seed Pvt. Ltd.) for





Fig. 2. Androgenesis in development of DHs via callus culture from a rice hybrid, BS6444G (A) Mid uninucleate stage of microspore, (B) Calli induction from pollens of anthers, (C) Initiation of green shoot buds from calli, (D) Green shoot regeneration (E) Well developed green shoots, (F) Rooting in microshoots, (G) Anther derived plants grown in net house, (H) Ploidy assessment of anther derived plants, (I) DHs in Field

generation of DHs (Rout et al. 2016; Naik et al. 2017) (Fig. 2). Further, anther culture was used for development of mapping population from Savitri (a high vielding indica rice variety) x Pokkali (a salt tolerant indica rice genotype) for identification of salt tolerant QTL/gene. However, the production of albinos (60-100%) in all the cultures proved to be detrimental for optimization of androgenic response. Therefore, NRRI attempted to develop a protocol for suppression of albinism which is also a frustrating feature in whole world. This led to standardization of 100% albino free shoot regeneration method in indica rice; patent filed 1355/KOL/2015 entitled "Method for albino free shoot regeneration in rice through anther culture". Subsequently, the improved protocol could generate 150, 200, 117, 73

and 30 DHs from CRHR32, BS6444G, Savitri x Pokkali, B x B and R x R respectively; surprisingly, no haploids were observed in BS6444G. Further, six promising DH lines each derived from CRHR32 and BS6444G showed at par yield with parent hybrid along with acceptable grain quality. Two promising DH lines of BS6444G showing at par yield over donor were found to be aromatic confirmed by PCR and sequencing of *badh2*. Moreover, four DH lines derived from rice hybrid, CRHR32, were found containing high protein (11.59 - 12.11%) in brown rice. Furthermore, iso-cytorestorer lines were developed through test cross of the 13 DHs (BS6444G) carrying positive *Rf4* genes with the CMS, with an average of 500-600 grains per panicle.

A systematic study with 117 DHs derived from  $F_1s$  of Savitri (popular HY rice variety) and Pokkali (salinity tolerant) could identify 4 candidate genes such as LOC\_Os01g09550 (no apical meristem protein), LOC\_Os01g09560 (mitochondrial processing peptidase subunit alpha), LOC\_Os12g06560 (putative protein) and LOC\_Os12g06570 (cyclic nucleotide-gated ion channel) for salinity tolerance at germination stage. In 2015, an efficient androgenic protocol was developed for another popular quality indica rice hybrid, 27P63 (M/S Pioneer, Hyderabad) and generated 315 green plants. After proper examination of ploidy status based on morphology, 246 plants were found to be diploids from which SSR markers identified a single heterozygote plant; this was also confirmed in the A1 generation. Then 245 DHs were advanced for further selection in identification of superior lines. A total of 170 DHs were selected to be promising based on agronomic traits in both the seasons, 2016-17 (A3 generation). Subsequently, application of the developed androgenic protocol could generate DHs from  $F_1$ s of Chakhao x IR20 which are being evaluated in field.



## 5.6. Field screening of genotypes for heat tolerance

Staggered sowing based screening for thermotolerance were performed for seven hundred genotypes and three genotypes (AC39843, AC39834 and AC39969) were found highly tolerant to heat stress with SES score 1 with more than 80% spikelet fertility; these genotypes showed at par /better yield than the check, Annapurna and N22. Besides, genotypes showing moderate degree of tolerance such as AC39975, AC11069, AC10925 and AC39935 were identified which showed 75% spikelet fertility under summer condition. Rice SNP seek database was used to identify the single nucleotide polymorphism present in the five rice OsHsfA2s (A2a, A2b, A2c, A2d, and A2d). The screening of filtered SNPs for these Hsfs identified on an average 11 SNPs for the five HsfA2 genes. Among them, HsfA2b was found to be highly conserved in rice with only two SNPs and HsfA2c showed maximum number of SNPs i.e. 16. But most of the SNPs were present in UTR or upstream or downstream region of these genes with only 1 or 2 SNPs resulted in non-synonymous substitution in the coding sequences of the rice HsfA2s. The position of non-synonymous substitution among the rice varieties were analyzed for comparison of divergence analysis between HsfA2 of different plants and the variation in rice. It showed, two non-synonymous substitution amino acids identified through divergence studies also showed divergence in different varieties of rice, i.e. arginine to isoleucine substitution in HsfA2d and arginine to histidine substitution in HsfA2e. The allele frequency of all the non-synonymous substitution were analyzed through SNP seek database to find out fixation of any alleles in any specific types of rice; it showed the frequency of all the non-synonymous substitution in OsHsfA2s was similar in all types of rice in comparison with reference allele except for the aspartate to glutamate substitution in HsfA2a. The non-reference allele frequency of this substitution were about 99.49% in aus type rice lines and 94.44% in aromatic collections of rice whereas in all other types of rice, the reference allele were found in higher frequency or at least equal frequency with non-reference allele, denoting there was no pattern involved for other nonsynonymous substitutions. Interestingly, all the dicots *HsfsA2* has glutamate as invariant at the respective position of the HsfA2. CRISPR/Cas9 work has been initiated at NRRI, and construct for editing of yield related genes was ready for transfer to some popular rice varieties.

## 6. KNOWLEDGE GAPS

- The recalcitrant nature of indica rice requires optimization of anther culture method which is very much important to achieve the potential yield of androgenesis technology in indica rice lines. Even though, attempt were made by Rout et al. (2016) and Naik et al. (2017) in these issues, additional novel attempts for media manipulation has to be addressed to increase the callusing potential or somatic embryogenesis.
- The identification of genomic regions that contribute to promising yield in rice from heterotic F<sub>1</sub> hybrids has to be addressed by analyzing the genetic structure



of DHs. Recent studies with *Arabidopsis* and maize (Wang et al. 2015) suggest that heterosis could be fixed by carefully combining genomic regions from the parents that contribute to better performance.

- The genes and pathways involved during pollen development in rice and characterization of heat stress responsive genes during anthesis and pollination would significantly increase the spikelet fertility during heat stress in rice. Additionally, strategies for robust phenotyping need to be developed for screening of large sets of accessions for heat stress tolerance.
- The efficiency of genetic transformation is very low in indica rice for which efficient transformation system in rice needs to be developed for successful generation of several transgenic rice plants.
- Though CRISPR/Cas9 is considered as the highly useful tool for genome editing, single base pair editing for altering one specific amino acids of a protein is very difficult for which genome editing for single base pair alterations needs to be standardized.

# 7. WAY FORWARD

There is a need to develop a novel media composition including plant growth regulators and histoneacetylase inhibitors to increase the callusing potential of the anthers from indica rice. Simultaneously, direct somatic embryogenesis from microspores needs to be focused as this method is considered as cost effective among all the pathways in tissue culture. Moreover, the mechanism of spontaneous chromosome doubling in androgenesis requires immediate attention. Additionally, the conceptual understanding of superior yield of DHs is still not clear. Thus, the scientific basis of superior yield of DHs needs to be comprehensively studied through generation of large number of DHs (~200 DHs from each hybrid). Further, high throughput genotyping and validation of identified genomic loci/superior alleles has to be evaluated in several genetic backgrounds and also through transgenic approaches. On the other hand, a highly robust genotype independent genetic transformation system for mega rice varieties has to be developed through combination of several factors in indica rice. Though the variability explained through mapping studies on heat stress tolerance is less than 30%, comprehensive phenotyping strategies necessitate to be employed for the identification of the complete genetic variation in tolerant cultivars. Simultaneously, heterologous expression of heat stress responsive genes requires to be worked out for enhancing the field tolerance to heat stress in rice. The field evaluation of rice transgenic lines should be promoted in institutes to understand the effect of transgenic rice in field condition. Specifically, the economic impact of cultivation of transgenic crops needs to be studied thoroughly to assess the potential of transgenic rice cultivation. Furthermore, it is required to standardize an efficient delivery system (Agrobacterium, biolistics, protoplast transfer) for CRISPR/Cas9 constructs to improve the efficiency of genetic modifications.



#### References

- Ball ST, Zhou HP and Konzak CF (1993) Influence of 2, 4-D, IAA and duration of callus induction in anther culture of spring wheat. Plant Science 90: 195-200.
- Bjornstad A, Opsahl-Ferstad HG and Aasmo M (1989) Effects of donor plant environment and light during incubation of anther cultures of some spring wheat (*Triticum aestivum L.*). cultivars. Plant Cell, Tissue and Organ Culture 17: 27-37.
- Blakeslee AF, Belling J, Farnham ME and Bergner AD (1922) A haploid mutant in the Jimson weed, Datura stramonium. Science 55: 646–647.
- Chandrasegaran S and Carroll D (2016) Origins of programmable nucleases for genome engineering. Journal of Molecular Biology 428: 963-989.
- Cruz RPD, Sperotto RA, Cargnelutti D, Adamski JM, FreitasTerra T and Fett JP (2013) Avoiding damage and achieving cold tolerance in rice plants. Food and Energy Security 2 (2): 96-119.
- Grewal D, Gill R and Gosal SS (2006) Role of cysteine in enhancing androgenesis and regeneration of *indica* rice. Plant Growth Regulator 49: 43-47.
- Grimes HD and Hodges TK (1990) The inorganic ratio influences plant regeneration and auxin sensitivity in primary callus derived from immature embryos of indica rice (*Oryza sativa* L.). Journal of Plant Physiology 136:362-367.
- Guha S and Maheshwarsi C (1964) In vitro production of embryos from anthers of *Datura*. Nature 204: 497.
- Guha S and Maheshwarsi C (1966) Cell division and differentiation of embryos in the pollen grains of *Datura* in vitro. Nature 212: 97-98.
- Hasan MM, Rafi MY, Ismail MR, Mahmood M, Rahimd HA, Alam MA, Ashkani S, Malek MA, and Latif MA (2015) Marker-assisted backcrossing: a useful method for rice improvement. Biotechnology and Biotechnological Equipment 29: 237–254.
- Herath HMI, Bandara DC and Samarajeewa PK (2007) Effect of culture media for anther culture of *indica* rice varieties and hybrids of *indica* and *japonica*. Tropical Agricultural Research and Extension 10: 17-22.
- Ishimaru T, Hirabayashi H, Sasaki K, Ye C and Kobayashi A (2016) Breeding efforts to mitigate damage by heat stress to spikelet sterility and grain quality. Plant Production Science 19: 12–21.
- Ivanova M and Van Staden J (2009) Nitrogen source, concentration, and NH<sub>4</sub><sup>+</sup>: NO<sub>3</sub><sup>-</sup> ratio influence shoot regeneration and hyperhydricity in tissue cultured *Aloe polyphyllaL*. Plant Cell, Tissue and Organ Culture 99(2): 167-174.
- Jagadish SVK, Craufurd PQ and Wheeler TR (2007) High temperature stress and spikelet fertility in rice (*Oryza sativa* L.) Journal of Experimental Botany 58: 1627–1635.
- Jagadish SVK, Murty MVR and Quick WP (2015) Rice responses to rising temperatures-challenges, perspectives and future directions. Plant, Cell and Environment 38: 1686–1698.
- Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA and Charpentier E (2012) A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science 337: 816.
- Kanda Y, Yokotani N, Maeda S, Nishizawa Y, Kamakura T, Mori M (2017) The receptor-like cytoplasmic kinase BSR1 mediates chitin-induced defense signaling in rice cells. Bioscience, Biotechnology and Biochemistry 81(8):1497-1502.
- Lee DK, Park SH, Seong SY, Kim YS, Jung H, Do Choi Y and Kim JK (2016) Production of insectresistant transgenic rice plants for use in practical agriculture. Plant Biotechnology Reports 10(6): 391-401.



- Ma X, Zhu Q, Chen Y and Liu YG (2016) CRISPR/Cas9 platforms for genome editing in plants: developments and applications. Molecular Plant 9: 961–974.
- Masuda H, Aung MS and Nishizawa NK (2013) Iron biofortification of rice using different transgenic approaches. Rice 6(1): 40.
- McCallum CM, Comai L, Greene EA and Henikoff S (2000) Targeted screening for induced mutations. Nature Biotechnology 18: 455–457.
- Minj AK, Sahu M, Chopkar R, Jha Z and Verulkar SB (2016) Optimization of media and cultural conditions for androgenesis in two *indica* rice crosses to regeneration doubled haploid plants. Journal of Cell and Tissue Research 16(3): 5899-5904.
- Mishra R, Rao GJN and Rao RN (2013) Effect of cold pretreatment and phytohormones on anther culture efficiency of two *indica* rice (*Oryza sativa* L.) hybrids: Ajay and Rajalaxmi. Journal of Experimental Biology and Agricultural Science 1(2): 69–76.
- Naik N, Rout P, Verma RL, Umakanta N, Katara JL, Sahoo K, Singh ON and Samantaray S (2016) Development of promising doubled haploids from indica rice hybrid: evaluation of uniformity and stability through morphological and molecular markers. International Journal of Agriculture Science 8(47): 1987-1992.
- Naik N, Rout P, Verma RL, Ngangkham U, Katara JL, Sahoo K, Singh ON and Samantaray S (2017) Development of Doubled Haploids from an elite indica rice Hybrid (BS6444G) using anther culture. Plant Cell, Tissue and Organ Culture 128: 679–689.
- Nieves-Cordones M, Mohamed S, Tanoi K, Kobayashi NI, Takagi K, Vernet A, Guiderdoni E, Périn C, Sentenac H and Véry AA (2017) Production of low-Cs+rice plants byinactivation of the K+transporter OsHAK1 with the CRISPR-Cas system. Plant Journal 92: 43–56.
- Niizeki H and Oono K (1968) Induction of haploid rice plant from anther culture. Proceeding of Japan Academy of Science 44: 554–557.
- Nurhasanah, Pratama AN, Rusdiansyah and Sunaryo W (2015) Effect of genotype and developmental stage of pollen on the success anther culture of local upland rice varieties from East Kalimantan. Asian Journal of Microbiology, Biotechnology and Environmental Science 17(2): 329-340.
- Prasad PVV, Boote KJ, Allen LH, Sheehy JE and Thomas JMG (2006) Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. Field Crops Research 95: 398–411.
- Qiu Z, Kang S, He L, Zhao J, Zhang S, Hu J, Zeng D, Zhang G, Dong G, Gao Z, Ren D, Chen G, Guo L, Qian Q and Zhu L (2017) The newly identified heat-stress sensitive albino1 gene affects chloroplast development in rice. Plant Sci. DOI: 10.1016/j.plantsci.2017.11.015.
- Reddy INBL, Kim BK, Yoon IS, Kim KH and Kwon TR (2017) Salt Tolerance in Rice: Focus on Mechanisms and Approaches. Rice Science 24(3): 123-144.
- Rout P, Naik N, Umakanta N, Verma RL, Katara JL, Singh ON and Samantaray S (2016) Doubled haploids generated through anther culture from an elite long duration rice hybrid, CRHR32: Method optimization and molecular characterization. Plant Biotechnology 30: 1-10.
- Senadhira D, Zapata-Arias FJ, Gregorio GB, Alejar MS, De La Cruz HC, Padolina TF, Galvez AM (2002) Development of the first salt tolerant rice cultivar through *indica/indica* anther culture. Field Crops Research 76(2/3): 103–110.
- Shanmugavadivel PS, Amitha Mithra SV, Prakash C, Ramkumar MK, Tiwari RK, Mohapatra T and Singh NK (2017) High resolution mapping of QTLs for heat tolerance in rice using a 5K SNP array. Rice 10: 28.
- Shen R, Wang L, Liu X, Wu J, Jin W, Zhao X, Xie X, Zhu Q, Tang H, Li Q, Chen L and Liu YG (2017) Genomic structural variation-mediated allelic suppression causes hybrid male sterility in rice. Nature Communication 8: 1310.



- Shi PH, Tang L, Wang LH, Sun T, Liu LL, Cao WX and Zhu Y (2015) Post-heading heat stress in rice of South China during 1981–2010. PLoS One 10(6):e0130642.
- Shimatani Z, Kashojiya S, Takayama M, Terada R, Arazoe T, Ishii H, Teramura H, Yamamoto T, Komatsu H, Miura K, Ezura H, Nishida K, Ariizumi T and Kondo A (2017) Targeted base editing in rice and tomato using a CRISPR-Cas9 cytidine deaminase fusion. Nature Biotechnology 35: 441–443.
- Sun YW, Jiao GA, Liu ZP, Zhang X, Li JY, Guo XP, Du WM, Du JL, Francis F, Zhao YD and Xia LQ (2017) Generation of high-amylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. Frontiers in Plant Science 8: 298–312.
- Teixeira E, Fischer G, van Velthuizen H, Walter C and Ewert F (2013) Global hot-spots of heat stress on agricultural crops due to climate change. Agricultural and Forest Meteorology 170: 206-215.
- Veeraraghavan R (2007) A study on the comparison of anther culture response in different varieties of rice, *Oryza sativa* subspecies *indica*. MSc thesis, University of Colombo.
- Wang L, Greaves IK, Groszmann M, Wu LM, Dennis ES and Peacock WJ (2015) Hybrid mimics and hybrid vigor in *Arabidopsis*. Proceeding of the National Academy of Science of the USA 112: E4959–E4967.
- Xie Y, Niu B, Long Y, Li G, Tang J, Zhang Y, Ren D, Liu YG and Chen L (2017a) Suppression or knockout of SaF/SaM overcomes the Sa-mediated hybrid male sterility in rice. Journal of Integrative Plant Biology 59: 669–679.
- Xie Y, Xu P, Huang J, Ma S, Xie X, Tao D, Chen L and Liu YG (2017b) Interspecific hybrid sterility in rice is mediated by OgTPR1 at the S1 locus encoding a peptidase-like protein. Molecular Plant 10: 1137–1140.
- Zhang P, Zhang Y, Sun L, Sinumporn S, Yang Z, Sun B, Xuan D, Li Z, Yu P, Wu W, Wang K, Cao L and Cheng S (2017) The Rice AAA-ATPase OsFIGNL1 is essential for male meiosis. Frontiers in Plant Science DOI: 10.3389/fpls.2017.01639.♥



# Development of Genomic Resources for Rice Improvement

### L Behera, C Parameswaran, A Anandan, P Sanghamitra, SK Pradhan, M Jena, N Umakanta, SK Dash, P Swain, RK Sahu, A Kumar, K Chattopdhyaya, J Meher, HN Subudhi, GP Pandi G and Devenna BN

## **1. INTRODUCTION**

Rice has rich genetic diversity in the form of thousands of landraces, elite breeding lines, high yielding varieties and 21 wild species. These differ tremendously in the levels of grain vield, quality of grains, input use efficiency, and tolerance to biotic and abiotic stresses with immense variation. Hence, rice consist of a rich source of naturally occurring alleles for the improvement of several traits including yield. The yield levels of rice varieties are greatly influenced by the environmental conditions and field management practices. There are remarkable interactions between genotypes and environments in such a way that varieties are adapted to specific environmental conditions. It is increasingly being recognized that exploitation of gene pools of rice germplasm is the fastest and acceptable approach to achieve the twin goals of high productivity and adaptability (Gur and Zamir 2004; Kovach and McCouch 2008). Development of high yielding varieties is possible by accumulation of beneficial and superior alleles from germplasm. More than 120,000 accessions of rice germplasm comprising traditional varieties, landraces, genetic stocks, breeding lines and wild relatives are preserved in gene banks at different places of India and other countries. Therefore, it is important to identify genes/QTLs for yield, quality traits, low input use efficiency, tolerance to biotic and abiotic stresses, etc from rice germplasm, and to introgress these into high yielding popular rice varieties through marker-assited selection (MAS). This would provide impetus to marker-assisted breeding on one hand and enable gene discovery on the other for sustainable agriculture, and improving rice yield potential (Ashikari and Matsuoka 2006). Realizing the immense potential of GENOMICS, concerted efforts are essential for yield improvement under changing climate conditions.

Sustained efforts have made possible to more than double the rice production since the green revolution in the 1960s. There has been a gradual decline in the annual growth rate of global rice production, while the population in rice-consuming countries is increasing at a rate of 1.8 percent per year. Increasing rice yield to ensure food security is a major challenge. Rice production is no longer keeping in pace with population growth during last decade because of shrinking cultivable land area, water scarcity, depletion of soil fertility, global warming (climate change), evolution of new biotypes, pathotypes, etc. Hence, it is necessary to use innovative tools to assist conventional methods in order to improve production and feed growing population. The availability of the complete genome sequence of rice and the



developments in the field of genomics has opened the door for speeding-up breeding processes for increasing yields and minimizing production risks. High throughput genomics is a promising approach in a holistic manner for mapping of genes/QTLs associated with target traits, gene prospecting and allele mining for useful traits, and re-sequencing of rice germplasm for discovery of SNPs, InDels, and precision breeding through MAS approach.

The objectives of the chapter are a) identification of genes/QTLs associated with resistance to brown plant hopper (BPH), pigmentation and antioxidants; b) association mapping to identify genes/QTLs for seedling vigor and tolerance to drought stress; c) gene prospecting and allele mining for tolerance to heat stress; and d) whole genome re-sequencing of donors and elite rice cultivars.

## 2. IDENTIFICATION OF GENES/QTLS ASSOCIATED WITH RESISTANCE TO BROWN PLANT HOPPER, PIGMENTATION AND ANTIOXIDANTS

#### 2.1. Brown plant hopper

Brown planthopper (BPH; Nilaparvata lugens Stal) is one of the most destructive insect pests in rice-growing areas of Asia and south-east Asia. Both adults and nymphs of the insect feed on rice sheaths by sucking sap from the phloem. All the growth stages of rice plant in the field are vulnerable to BPH. Mild infestation leads to yellowing of leaves, reduction in plant height, growth, vigor, number of productive tillers and grain filling. Heavy infestation causes complete drying and death of plants, a condition known as "hopperburn" (Sogawa 1982; Watanabe and Kitagawa 2000). Brown planthopper also transmits rice tungro, grassy stunt and rugged stunt viruses and causes indirect damage to rice plant (Ling et al. 1978; Hibino 1996; Rivera et al. 1996). The frequency of outbreaks and severity of damage have increased since 1990s because of year-round cultivation of semi-dwarf, photo-period insensitive, genetically homogeneous varieties with greater use of fertilizers and insecticides. Use of resistant varieties is one of the best options to reduce the BPH damage. Systematic breeding programs have led to the identification of several donors, which have been used to develop BPH resistant varieties and to identify genes/QTLs associated with BPH resistance. Brown planthopper resistance is a complex trait governed by both major and minor genes. Thirty-two major BPH resistance genes have been identified using classical genetics and molecular approaches (Jena and Kim 2010; Fujita et al. 2013; Wu et al. 2014; Wang et al. 2015a; Hu et al. 2016; Prahalada et al. 2017). These genes have been mapped to six rice chromosomes 2, 3, 4, 6, 11 and 12. Eighteen genes, Bph1, bph2, Bph3, Bph6, Bph9, bph12, Bph14, Bph15, Bph17, Bph18, bph19, Bph 25, Bph26, Bph27, Bph28, bph29, Bph30 and Bph32 have been fine-mapped. In addition to major genes, many QTLs associated with BPH resistance have been identified using different mapping populations and screening parameters (Jena et al. 2010; Fujita et al. 2013; Deen et al. 2017; Mohanty et al. 2017). The major genes Bph1, bph2, Bph3, Bph14, Bph15, Bph18 and QTLs QBph3 and QBph4 have been used for introgression into elite rice cultivars through marker-assisted



breeding approach (Sharma et al. 2004; Jairin et al. 2009; Suh et al. 2011; Hu et al 2012; 2013; 2015; 2016). Xiao et al. (2016) individually transferred 13 genes/QTLs, *Bph14, QBph3, QBph4, Bph17, Bph15, Bph20, Bph24, Bph6, Bph3, Bph9, Bph10, Bph18* and *Bph21* into cultivar 93-11 by marker-assisted backcross breeding (MABB). The NILs were evaluated against BPH. All genes reduced BPH growth, development, and showed antibiotic responses in seedlings. Only seven resistance genes, namely, *Bph3, Bph9, Bph14, Bph18, Bph26, bph29* and *Bph32* have been cloned (Du et al. 2009; Tamura et al. 2014; Liu et al. 2015; Wang et al. 2015a; Ji et al. 2016; Ren et al. 2016; Zhao et al. 2016b) (Table 1).

S. No	. Gene	Protein product	Function	References
1	Bph3	Plasma membrane- localized lectin receptor kinases.	Play a critical role in priming the pattern-triggered immunity response to BPH infestation by perceiving herbivore-associated molecular patterns (HAMPs) or damage-associated molecular patterns (DAMP),or mediating the downstream signaling events.	Liu et al. 2015
2	Bph9	Nucleotide-binding and leucine-rich repeat (NLR) containing protein.	Activates salicylic acid and jasmonic acid-signaling pathways and confers both antixenosis and antibiosis to BPH.	Zhao et al. 2016b
3	Bph14	Coiled-coil nucleotide-binding and leucine-rich repeat (CC-NBS- LRR) protein.	Mediates resistance mechanism through activation of salicylic acid(SA) signaling pathway and induces callose deposition in phloem tissue that inhibits BPH feeding on the host plant.	Du et al. 2009
4	Bph18	Coiled-coil nucleotide-binding and leucine-rich repeat (CC-NBS- LRR) protein.	Proteins are widely localized to the endo-membranes in a cell, including the endoplasmic reticulum, Golgi apparatus, trans- Golgi network, prevacuolar compa and recognize the BPH invasion at endo-membranes in phloem cells.	Ji et al. 2016 rtments
5	Bph26	Coiled-coil nucleotide-binding and leucine-rich repeat (CC-NBS- LRR) protein.	Mediate sucking inhibition in the phloemsieve element.	Tamura et al. 2014
6	bph29	B3 DNA-binding domain protein.	Confers BPH resistance through activation of SA pathway and suppression of jasmonic acid/ ethylene-dependent pathway.	Wang et al. 2015a
7	Bph32	A short consensus repeat (SCR) domain protein.	The protein is localized in the plasma membrane of the cell and confers an antibiosis resistance to BPH.	Ren et al. 2016

#### Table 1. Examples of cloning of BPH resistance genes in rice.



The primary sources of resistance to BPH have been reported from different centers in India. Jena et al. (2002) identified OPA16,938 RAPD marker linked to BPH resistance present in IR54741- 3-21-22, an introgression line from Oryza officinalis. Soundararajan et al. (2004) mapped six QTLs associated with seedling resistance, antibiosis and tolerance on chromosomes 1, 2, 6, and 7 for BPH resistance using a DH population (IR64 × Azucena). Genetics of BPH resistance revealed the presence of two major genes, Bph3 and Bph6 in the donors Velluthacheera, T1471, T1426 and T1432; Bph6 gene in ARC14529, ARC14771 and IR72; bph4 gene in ARC5984, Manoharsali and Sonasali (Padmavathi et al. 2005). Deen et al. (2010) identified 3 BPH resistance genes *i.e.*, *Bph22(t)*, *Bph23(t)* and *bph24(t)* from *O. gaberrima*, *O. minuta* and O. rufipogon, respectively. Kumari et al. (2010) reported that two markers, RM3180 and RM2453 on chromosome 3 were linked with BPH resistance using a RIL population derived between IR50 and Rathu Heenati. Deen et al. (2017) identified five major QTLs associated with BPH resistance, aBphDs6 for damage score, aBphNp(48h)-1 and *qBphNp(72h)-12* for nymphal preference, and *qBphDw(30)-3* and *qBphDw(30)-8* for days to wilt contributing phenotype variance of 24.23, 8.69, 7.66, 4.55 and 10.48%, respectively.

Behera et al. (2012) assessed the genetic relationship among 19 rice cultivars differing in resistance to BPH using 42 SSR markers. Genetic similarities among cultivars varied from 0.38 to 0.897 with an average of 0.604. Seven unique alleles were identified. Jena et al. (2015) genotyped 48 rice cultivars including 39 landraces using 22 genelinked markers of BPH resistance. Genetic diversity analysis categorized all the genotypes into 4 major clusters with the 40% level of genetic similarity. Mohanty et al. (2017) identified two QTLs,

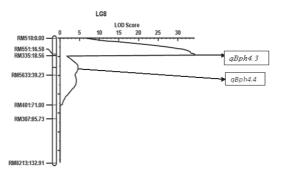


Fig. 1. Two QTLs *qBph4.3* and *qBph4.4* on linkage group8 (Chromosome 4) for BPH resistance were identified in the resistant rice cultivar Salkathi. Left side of graph shows SSR markers and their positions in cM. LG8 represents short arm of chromosome 4. Right side of graph shows two peaks corresponding QTLs *qBph4.3* and *qBph4.4* with LOD score of 34.2 and 4.61, respectively. The *qBph4.3* is a novel QTL. Source: Mohanty et al. (2017).

*qBph4.3* and *qBph4.4* linked to BPH resistance in resistant cultivar Salkathi (Fig. 1). The *qBph4.3* seems to be a novel QTL associated with BPH resistance. Brown planthopper resistance in Sakathi has been successfully transferred into two elite rice cultivars, Pusa 44 and Samba Mahsuri. The promising resistant breeding lines developed from these varieties have been validated for the presence of these QTLs using linked markers. Further, work is in progress for fine mapping and transfer of these QTLs into elite susceptible cultivars, Pooja and Swarna.



#### 2.2. Anthocyanin and antioxidants

Colored rice genotypes (red, brown, purple, black) are the rich source of dietary fiber, vitamins, phytic acids and phytochemicals such as phenolics (á-tocopherols, tocotrienols and a-oryzanol) and flavonoids (anthocyanins, proanthocyanidins). These compounds are good source of natural antioxidants and grain color, which help in decreasing the toxic compounds, oxidative stress and reduce the risk of cardiovascular disease, type-2 diabetes, and also prevention of some cancers (Hudson et al. 2000; Cicero and Gaddi 2001; Ling et al. 2001; Hu et al. 2003; Xia et al. 2003; Liu 2007; Yawadio et al. 2007; Arab et al. 2011; Walter et al. 2013). The colored genotypes contain higher amounts of phytochemicals than non-pigmented genotypes (Goffman and Bergman 2004; Shen et al. 2009). Anthocyanins and proanthocyanidins are the primary pigments in colored rice. Among colored rice, black rice genotypes exhibit the highest antioxidant activities followed by purple, red, and brown rice. The colour of purple and black grains is due to the presence of anthocyanins (Reddy et al. 1995). The phenolic compounds are mainly associated with the pericarp colour. The darker the pericarp, higher the amount of polyphenols (Tian et al. 2004; Zhou et al. 2004; Yawadio et al. 2007). The insoluble compounds appear to constitute the major fraction of phenolic acids and proanthocyanidins in rice, but not flavonoids and anthocyanins. Hence, rice should be preferentially consumed in the form of bran or as whole grain to maximize the intake of antioxidant compounds. Phytic acid is vital for seed development and higher seedling vigor. It is often considered as an anti-nutritional substance but has the positive nutritional role as an antioxidant, anti-cancer agent, and lowering heart and coronary diseases in humans (Bohn et al. 2008; Gemede 2014). The japonica rice varieties are found to be richer in antioxidant compounds compared with indica rice varieties. Fasahat et al. (2012) reported higher nutritional and antioxidant properties of whole grains of O. rufipogon.

Two loci, *Rc* and *Rd* are involved in proanthocyanidin synthesis in rice pericarp. When present together, these two loci produce red seed color. Rc produces brown seeds in the absence of Rd, whereas Rd alone has no phenotype. Both genes have been cloned and sequenced (Sweeney et al. 2006; Furukawa et al. 2007). Cyanidin-3glucoside and peonidin-3-glucoside are the two main pigments deposited in grain pericarp of black rice (Abdel-Aal et al. 2006). Two loci, Pb (Prp-b) and Pp (Prp-a), located on chromosome 4 and 1, respectively are required for the pericarp pigmentation with anthocyanins of black rice (Yoshimura et al. 1997). Wang and Shu (2007) mapped *Pb* gene on rice chromosome 4 responsible for purple color. Tan et al. (2001) identified three, two and four QTLs for the color parameters of lightness (L), redness (a) and yellowness (b), respectively. Jin et al. (2009) reported several QTLs responsible for brown rice color, total phenolic and flavonoid contents in rice grain using a doubled haploid population. Shao et al. (2011) identified 41 markers significantly associated with QTLs for grain color and nutritional quality traits using 416 rice accessions including red and black rice. Their study indicated that *Ra* (*Prp-b* for purple pericarp) and Rc (brown pericarp and seed coat) genes control rice grain color and nutritional quality traits. Maeda et al. (2014) identified three loci, Kala1, Kala3 and Kala4 on



chromosomes 1, 3 and 4, respectively, which are associated with black pigmentation. They introduced these loci into Koshihikari, a leading variety of Japan from black rice, Hong Xie Nuo. Xu et al. (2016) identified loci for phenolic related traits and one locus for ferulic acid in 32 red and 88 white pericarp accessions of rice using SNP markers. Xu et al. (2017) identified 21 additive QTLs for anthocyanins (ANC) and proanthcyanidins (PAC) using RIL population developed from red rice Hong Xiang1 (HX1) and white rice Song 98-131 (S98-131). Two new QTLs, *qANC3* and *qPAC12-4* were detected in several environments and explained significant phenotype variance.

The anthocyanin and gamma-oryzanol content of 160 pigmented rice genotypes was evaluated by Sanghamitra et al. (2017). The purple grain type genotypes, namely, Kalobhat, Mamihunger, Chakhao, Manipuriblack and Kalabiroin were identified with rich source of anthocyanin content whereas Mamihunger, Chakhao and Kalobhat were observed with rich source of gamma-oryzanol content compared to red and brown grain type. Fate of anthocyanin and gamma-oryzanol content were also assessed after processing, cooking and in different end user products of three pigmented rice genotypes such as Chakhao, Mamihunger and Mornodoiga. The highest reduction of anthocyanin and gamm-aoryzanol content (97% and 88%, respectively) was observed in parboiled rice, whereas only 2-3% reduction in anthocynin and 70% reduction in gamma-oryzanol content was observed in end use products like in popped and puffed rice. Further, work is continuing to identify QTLs associated with anthocyanin and antioxidants using RIL population developed from colored and non-colored rice genotypes.

# 3. ASSOCIATION MAPPING TO IDENTIFY GENES/QTLS FOR SEEDLING VIGOR AND TOLERANCE TO DROUGHT STRESS

Association mapping is a powerful and promising approach for identification and mapping of genes/QTLs associated with phenotypic traits in living organisms (Hall et al. 2010; Stich and Melchinger 2010). This approach is increasingly being used in plant species, e.g. maize, rice, barley, wheat, sorghum, sugarcane, sugar beet, Arabidopsis, potato, soybean, grape, forest tree species and forage grasses (Abdurakhmonov and Abdukarimov 2008). In association mapping approach, only polymorphisms with extremely tight linkage to a locus that causes the phenotype effect are likely to be significantly associated with the trait in a randomly mating population, thus providing a much finer resolution than bi-parental mapping. Conventional linkage analysis requires mapping populations (derived from a biparental cross) that are difficult to develop and is time consuming. Association mapping exploit the linkage disequilibrium (LD) already present in the natural population of interest, allows a much higher resolution, permitting survey of large number of alleles per locus, and has great potential for future trait improvement and germplasm security. Several researchers have used association mapping approach for identification of QTLs associated with different traits in rice, viz., grain yield and related traits (Agrama et al. 2007; Fei-fei et al. 2016; Zhang et al. 2017), agronomic traits (Huang et al. 2010;



Zhao et al. 2011; Zhou et al. 2012; Lu et al. 2015; Yano et al. 2016), stigma and spikelet characteristics (Yan et al. 2009), flowering time and grain quality (Huang et al. 2012), panicle architecture and spikelet's/panicle (Rebolledo et al. 2016), chlorophyll content (Wang et al. 2015b), mesocotyl elongation (Wu et al. 2015), harvest index (Li et al. 2012a), leaf traits (Yang et al. 2015), seedling vigor (Anandan et al. 2016), amylose contents (Jin et al. 2010), mineral element contents in whole grain (Huang et al. 2015), aluminum tolerance (Famoso et al. 2011), salinity tolerance (Kumar et al. 2015), cold tolerance (Pan et al. 2015; Pandit et al. 2017) and high temperature tolerance (Pradhan et al. 2016).

### 3.1. Seedling vigor

The weeds are the major bottleneck and cause yield reduction up to 48%, 53% and 74% in transplanted, direct-seeded flooded and aerobic rice, respectively. Further, manual weeding or spraying of herbicides incur additional cost of expenditure in rice cultivation. However, application of herbicides to control weeds have been proven to be effective, but in many cases, the intensive use may cause hazardous effect on environment and possibility of development of herbicide tolerance in weeds. On the other hand, the use of herbicides during monsoon time affects their efficiency. Therefore, the use of weed-competitive varieties to suppress weeds might substantially reduce herbicide use and labor cost. The rapid uniform germination and accumulation of biomass during initial phase of seedling establishment is an essential phenotypic trait considered as early seedling vigor for direct seeded situation in rice irrespective of environment (Mahender et al. 2015). Early seedling vigor (ESV) trait has been exploited in rainfed upland cultivar as those varieties are preferred over the others as they have weed smothering effect. The most of high yielding rice varieties for irrigated ecosystem are not suitable for dry direct seeded conditions as they are semi-dwarf in stature with reduced seedling vigor (Mahender et al. 2015; Anandan et al. 2016). Therefore, breeding rice varieties for direct seeded system combining high yield, early vigor and strong weed competitiveness is very much necessary. Several QTLs for seedling vigor and related traits have been reported in rice using bi-parental mapping populations and well reviewed by Mahender et al. (2015). Recently, association mapping strategies have been employed to identify QTLs for seedling vigor using natural populations of rice (Dang et al. 2014; Wu et al. 2015; Anandan et al. 2016; Lu et al. 2016). Dang et al. (2014) identified 18 SSR markers for three traits such as root length, shoot length and shoot dry weight associated with seed vigor using 540 rice cultivars and 262 microsatellite markers. Cheng et al. (2015) observed significant natural variation of seed germination and seedling growth among 276 accessions under normal, drought and salt conditions. A total of 12, 14 and 9 simple sequence repeat (SSR) markers associated with three traits were identified under normal, drought and salt conditions, respectively using association mapping approach. Wu et al. (2015) identified 13 loci associated with mesocotyl lengths of seedlings grown in water in darkness and three loci associated mesocotyl lengths grown in 5 cm sand culture using SNP array (Rice SNP50). Alpha-amylase precursor and ethyleneinsensitive3 are the genes found to be there in coding region. On the other hand, a gene OsGA20ox1 was found to be associated with gibberellin (GA) biosynthesis



from the fine mapping of major QTL *qPHS3-2*, which accounts for 26.2% phenotypic variance (Abe et al. 2012). These promising QTLs and candidate genes associated with seedling vigor would be useful for improving seedling vigour in high yielding popular rice varieties by introgressing them through marker-assisted breeding technique. Nagavarapu et al. (2017) validated the reported QTLs for seedling vigor in a set of 47 *indica* cultivars. Six out of eight QTLs, *qGR-1*, *qGP-6*, *qFV-3-2*, *qFV5-2* and *qFV-10* were detected in genotypes showed high seedling vigor. The genotypes Dinesh, Pooja and Sabita showed the presence of multiple QTLs indicating that these genotypes have inherent capacity for early emergence with high seedling vigor.

Anandan et al. (2016) identified 16 SSR markers which were significantly associated with early seedling vigor traits in 96 rice genotypes using association approach. These genotypes were selected from 629 rice accessions based on their morphological and physiological responses grown in the field under direct seeded aerobic situation. Further, they have reported the pleiotropic effect of marker RM341 on chromosome 2 for shoot dry weight on 28 DAS, vigor index on 14 and 28 DAS. Pandit et al. (2017) conducted association mapping of cold tolerance using a panel of 66 rice genotypes, and 58 SSR markers and 2 direct linked markers. These genotypes were selected based on the screening of 304 *indica* rice germplasm to seedling stage chilling tolerance. They identified nineteen SSR markers significantly associated with chilling stress tolerance at 8 °C to 4 °C for 7–21 days duration. The QTLs identified to cold tolerance, *qCTS9*, *qCTS-2*, *qCTS6.1*, *qSCT2*, *qSCT11*, *qSCT1a*, *qCTS-3.1*, *qCTS11.1*, *qCTS12.1*, *qCTS-1b*, and *qCTB2* would be useful in molecular breeding program for development of strongly chilling tolerant varieties.

#### 3.2. Drought tolerance

There are several successful examples of identification of QTLs/genes for drought tolerance using bi-parental mapping populations, but a few examples are available using GWAS approach in rice (Courtois et al. 2013; Lou et al. 2015; Al-Shugeairy et al. 2015; Muthukumar et al. 2015; Ma et al. 2016; Phung et al. 2016; Swamy et al. 2017). Courtois et al. (2013) identified 19 associations for deep root mass and the number of deep roots in 168 traditional and improved *japonica* accessions using GWAS approach. Al-Shugeairy et al. (2015) genotyped 371 cultivars of the Rice Diversity Panel using Affymetrix SNP array containing 44,100 SNPs and identified one significant association on chromosome 2 for drought recovery. Lou et al. (2015) performed genome-wide association study and identified six QTLs linked to deep rooting for drought avoidance in 180 recombinant inbred lines and an association mapping population containing 237 rice varieties using 10,19,883 SNPs. Phung et al. (2016) identified two associations for root thickness and crown root number by genotyping 180 rice accessions of Vietnam with 22,000 single-nucleotide polymorphism. Ma et al. (2016) identified 18, 5, and 6 loci associated with plant height, grain yield per plant, and drought resistant coefficient, respectively in 270 rice landraces and cultivars under contrasting moisture conditions using GWAS approach.

Few association analysis studies have been reported in rice in India. Sing et al. (2017) identified several QTL for early vigor and 8 related traits using 194 SNP markers



and BILs derived from cross between Swarna and Moroberekan. Six genomic regions containing QTLs for seedling vigor and related traits were identified. The QTLs located in two QTL hotspot regions on chromosome 3 and 5 were expressed consistently in field as well as glasshouse conditions. The majority of QTLs were clustered on chromosome 3 ( $qEV_{3,P}$ ,  $qEUE_{3,P}$ ,  $qSHL_{3,P}$ ,  $qSFW_{3,P}$ ,  $qTFW_{3,P}$ ,  $qRDW_{3,P}$ ) and chromosome 5 ( $qEV_{5,P}$ ,  $qEUE_{5,P}$ ,  $qSHL_{5,P}$ ,  $qSFW_{5,P}$ ,  $qSDW_{5,P}$ ,  $qTDW_{5,P}$ ). Muthukumar et al. (2015) studied marker–trait associations using 1168 SSR markers and 911,153 SNPs in 17 diverse rice lines from different geographical regions and hydro-logical habitats. They identified 23 consistent associations with drought tolerance traits. Swamy et al. (2017) genotyped 75 rice accessions with 119 highly polymorphic SSR markers and identified 80 marker-trait associations were identified (GY), plant height (PH) and days to flowering (DTF). Seven associations were identified for GY under drought stress. Most of these associations identified were on chromosomes 2, 5, 10, 11 and 12 and their phenotypic variance varied from 5 to 19%.

Massive screening work has been carried out to identify rice cultivars tolerant to drought stress at vegetative stage at ICAR-National Rice Reseach Institute, Cuttack. Screening of about 10,000 rice cultivars led to the identification of more than 250 tolerant genotypes at vegetative stage drought stress. Further, 384 genotypes including 100 tolerant cultivars at vegetative stage drought stress were evaluated under reproductive stage drought stress. An association mapping panel comprising of 285 genotypes was developed based on the grain yield under reproductive stage drought stress. Precise phenotyping of this association mapping panel genotypes was carried out during *Kharif* 2016 and *Rabi* 2017. Further, work is in progress for grain yield and related traits under reproductive stage drought stress. Two RIL mapping populations have been developed from the tolerant cultivars, Sahabhagidhan and Kalakeri, and susceptible cultivar IR20. The identification of QTLs under reproductive stage drought stress is in progress.

# 4. GENE PROSPECTING AND ALLELE MINING FOR TOLERANCE TO HEAT STRESS

Allele mining is the identification of nucleotide polymorphisms in the sequences of interest genes to understand the diversity, evolutionary significance, effect on the protein sequence and its functional impact on the organisms. This approach can be effectively used for discovery of superior alleles through mining the gene of interest from diverse genetic resources and development of allele-specific markers for use in the marker-assisted selection. Next generation sequencing technologies has provided enormous data of sequences of rice varieties. The sequences can be searched for identification of homologous genes and its sequence variation. There are various methods to identify the novel alleles of genomic regions. These include, amplification of targeted region and sequencing through Sanger's method of sequencing, TILLING/ EcoTILLING approaches, whole genome re-sequencing approaches, etc. All the novel variants identified might not cause functional change in the organisms because most of them would be occurring in the non-coding regions of the gene or could be



synonymous substitutions. Thus, allele mining of genes within the coding and promoter region could provide important information about the novel alleles of genes in rice. Several genes have been identified related to grain yield, morphological and quality traits, and tolerance to biotic and abiotic stresses. The allele mining studies have been reported for a number of genes in rice (Table 2). So far, only one gene *Thermotolerant 1* has been reported to have novel allele conferring increased tolerance to heat stress in rice. Thus, there is need to identify novel alleles for heat stress tolerance in rice.

S.	No.	Gene/QTL	Novel alleles/ haplotype	Efficient Source	Phenotypic effect	References
	1	PsToll	H17	O. rufipogan	Increased shoot and root length.	Neelam et al. 2017
	2	HKT1:5	7 major and 3 minor alleles	Aromatic lines	Higher Na <sup>+</sup> exclusion.	Platten et al. 2013
	3	Sub1A	INDEL	Meghi	Stronger post submergence recovery.	Goswami et al. 2017
4	4	TT1	Non-synony- mous substitution	Oryza glabberima	Increased spikelet fertility.	Li et al. 2015
:	5	DREB1A, DREB1B	Alleles in 5' UTR	-	No association with cold tolerance.	Challam et al. 2015
(	5	Pi54	9 new alleles	Group of genotypes	Varied patterns of resistance.	Vasudevan et al. 2015
1	7	Pi9	5 haplotypes	Landraces	Not performed.	Imam et al. 2016
8	3	Pid3	6 haplotypes	Indica	Similar resistance spectrum.	Lv et al. 2017
9	9	Xa21, Xa26, xa5	2 alleles	O. nivara	Effective resistance source.	Bimolata et al. 2015
	10	Xa13	18 haplotypes	-	Not performed.	Yu et al. 2016
	11	Ghd7, Ghd8, Hd1	10-21 haplotypes	Combination of <i>indica</i> , <i>japonica</i> and <i>Oryza</i> <i>rufipogan</i> alleles are photosensitive.	A combination of strong, weak and non functional effect on heading date, SSF for tropical condition.	Zhang et al. 2015
	12	DEP1	7 haplotypes	Japonica varieties.	Hap2 increased number of primary and secondary branches.	Zhao et al. 2016a
	13	Gn1a, OsSPL14, SCM2, Ghd7,DEP1, SPIKE,GS5, TGW6	2-5 haplotypes	Habataki, ST12, ST6, Aikawa1, YP9, Osmancik-97 and Kasalath.	Enhanced yield.	Kim et al. 2016

#### Table 2. Examples of allele mining of major genes in rice.



Heat stress is one of the important abiotic stresses which cause spikelet sterility up to 60% in rice during the flowering stage. Hence, it is considered as one of the major threat to crop production (Teixeira et al. 2013). Rice is sensitive to heat stress during gametogenesis and flowering stages. The cardinal temperature for heat stress in rice is >35 °C (Prasad et al. 2006). It is reported that there would be negative impact on rice yield due to warmer regime in future (Welch et al. 2010). Spikelet fertility under heat stress is considered as the major trait for evaluating the response of rice genotypes to heat stress. Nagina22 was identified as highly tolerant cultivar for heat stress (Prasad et al. 2006; Pradhan et al. 2016). Some of the *Oryza glabberima* accessions such as CG14 was also found to be tolerant because of early anthesis and pollination during dawn compared to *indica* cultivars (Jagadish et al. 2008).

Many QTLs have been identified for heat stress tolerance in rice using  $F_2$ , BIL and RIL populations (Cao et al. 2003; Chang-Lan et al. 2005; Chen et al. 2008; Zhang et al. 2008; 2009; Jagadish et al. 2010; Xiao et al. 2011a; 2011b; Cheng et al. 2012; Ye et al. 2012; 2015; Buu et al. 2014; Tazib et al. 2015; Zhao et al. 2016c). These mapping populations have been phenotyped at the time of heading in controlled environment conditions or under high temperature condition by late planting in open field.

Poli et al. (2013) identified significant association of RM1089 with number of tillers and yield per plant, RM423 with leaf senescence, RM584 with leaf width and RM229 with yield per plant using  $F_2$  population developed from IR64 and NH219 (N22-Hdgl219). NH219 is a dark green leaf mutant of N22 (N22-H-dgl219), which showed reduced accumulation of reactive oxygen species in leaf under 40°C heat conditions. Prashant et al. (2016) evaluated fixed breeding lines for heat stress tolerance in field condition. They indicated that spikelet sterility and yield per plant have to be taken as a criteria for evaluating heat stress tolerant rice lines. The phenotypic variance of the identified QTLs varied from 11% to 25% for spikelet fertility during heat stress in rice. Shanmugavadivel et al. (2017) identified five QTLs on chromosomes 3, 5, 9 and 12 for yield and percent spikelet sterility using 5K SNP array and RIL mapping population developed from N22 and IR64. These QTLs explained phenotypic variation in the range of 6.27 to 21.29%.

Pradhan et al. (2016) used association mapping approach to identify markers associated with high temperature stress tolerance. A set 60 genotypes were selected from 240 germplasm lines based on spikelet fertility percent under high temperature. These lines were genotyped with two INDEL and 18 SSR linked markers. The marker RM547 was associated with spikelet fertility while the markers like RM228, RM205, RM247, RM242, INDEL3 and RM314 indirectly controlling the high temperature stress tolerance were detected. A non-synonymous substitution in the *HsfA2a* gene in rice was found to be specific to Aus ecotypes in rice (NRRI, Annual Report 2016).

# 5. WHOLE GENOME RE-SEQUENCING OF DONORS AND ELITE RICE CULTIVARS

The availability of the complete rice genome sequence (Goff et al. 2002; Yu et al. 2002) and the advancement of next generation sequencing (NGS) technologies have



provided opportunity for sequencing of germplasm, discovery of genome-wide DNA variations such as SNPs and InDels, and mining information about diversity of genes and alleles (Bentley 2006; Varshney et al. 2009; Davey et al. 2011; Gao et al. 2012). These DNA sequence level variations can be associated with traits, and elucidate genomic structure and composition (McNally et al. 2009; Chen et al. 2014). Millions of DNA polymorphisms, including single nucleotide polymorphisms (SNPs), insertionand-deletion (InDels) and structural variant polymorphisms have been identified in rice germplasm by using high-throughput sequencing methods and bioinformatic tools (Table 3). The first SNP resource was developed based on the draft genome sequences of the *japonica* and *indica* rice cultivars (Feltus et al. 2004). Numerous DNA polymorphisms were identified by whole genome re-sequencing of a high quality japonica variety Koshikari (Yamamoto et al. 2010); Omachi, a Japanese landrace used for sake brewing (Arai-Kichise et al. 2011); restorer lines (IR24, MH63 and SH27)(Li et al. 2012b); elite *indica* rice inbred lines (three CMS and three R lines) (Subbaiyan et al. 2012), two Korean rice varieties (Hwayeong and Dongjin) and three anther-derived lines (BLB, HY04 and HY08) (Jeong et al. 2013), maintainer line (V20B) (Hu et al. 2014), seven cultivated temperate and tropical japonica groups (Arai-Kichise et al. 2014), two Korean *japonica* rice varieties (Junam and Nampyeong) (Jeong et al. 2015); northern japonica rice variety Longdao24, and its parents Longdao5 and Jigeng83 (Jiang et al. 2017) and salt tolerant rice cultivar SR86 (Chen et al. 2017). Li et al. (2014) re-sequenced 3000 rice accessions collected from 89 countries and identified 18.9 million SNPs

The NGS techniques has been used for QTL mapping associated with different traits, positional cloning, haplotype analysis and identification of seed purity of rice varieties (Feltus et al. 2004; McCouch et al. 2010; Shen et al. 2010; Chen et al. 2014). The genome-wide association studies for identification of QTLs associated with agronomic and yield related traits have been carried out based on the genome resequencing and SNP arrays (Huang et al. 2010; Zhao et al. 2011; Han and Huang 2013). Yang et al. (2017) used bulked segregant analysis combined with whole genome re-sequencing technology to map quantitative trait loci (QTL) and candidate genes for nitrogen use efficiency.

Jain et al. (2014) identified a total of 17,84,583 SNPs and 1,54,275 InDels by resequencing of three rice cultivars (IR64-drought sensitive, Nagina22-drought tolerant and Pokkali-salinity tolerant). Some of SNPs were identified in the differentially expressed genes within known QTLs. The whole genome re-sequencing of high yielding rice variety Swarna helped to understand genetic basis of low glycemic index (Rathinasabapathi et al. 2015). Rathinasabapathi et al. (2016) re-sequenced Kavuni, a traditional rice cultivar with nutritional and therapeutic properties, and identified 11,50,711 SNPs. Pathway mapping of these polymorphisms revealed the involvement of genes related to carbohydrate metabolism, translation, protein-folding and cell death. Analysis of the starch biosynthesis related genes revealed that the granulebound starch synthase I gene had T/G SNPs at the first intron/exon junction and a two-nucleotide combination, which were reported to favour high amylose content and low glycemic index.



SIN	Io. Genotypes Re-sequenced	DNA Polymorphism detected	References
1	20 diverse rice cultivars.	160,000 SNPs were identified, and revealed the breeding history and relationships among the 20 rice cultivars.	McNally et al. 2009
2	Omachi, a landrace of <i>japonica</i> rice, which is an important source for modern cultivars.	132,462 SNPs and 35,766 IDELs were identified between the Omachi and Nipponbare genome.	Arai-Kichise et al. 2011
3	50 rice cultivars(40 cultivated and 10 wild progenitors, <i>Oryza rufipogon</i> and <i>Oryza</i> <i>nivara</i> ).	6.5 million SNPs were identified. Significantly lower diversity was observed in cultivated rice as compared to wild rice.	Xu et al. 2012
4	6 elite <i>indica</i> rice inbred lines (three CMS and three R lines).	2,819,086 DNA polymorphisms were between the inbreds and Nipponbare.	Subbaiyan et al. 2012
5	3 important restore lines IR24, MH63 and SH27.	Numerous SNPs, InDels and structural variations were identified. The results showed higher genetic variations among restorer lines.	Li et al. 2012b
6	5 Korean rice accessions, including three anther culture lines (BLB, HY-04 and HY-08), their progenitor cultivar (Hwayeong), and an additional <i>japonica</i> cultivar (Dongjin).	1,154,063 DNA polymorphisms were detected. Higher polymorphism was found in anther culture derived li	
7	6 cultivars including 5 temperate <i>japonica</i> cultivars and 1 tropical <i>japonica</i> cultivar (Moroberekan).	Several of SNPs and InDels were identified.	Arai-Kichise et al. 2014
8	A maintainer line V20B.	660,778 SNPs and 266,301 InDels were identified with respect to <i>indica</i> reference genome 93-11.	Hu et al. 2014
9	3000 rice accessions collected from 89 countries.	Identified 18.9 million SNPs.	Li et al. 2014
10	Three cultivars (IR64-drought sensitive, Nagina22-drought tolerant and Pokkali-salinity tolerant).	17,84,583 SNPs and 1,54,275 InDels were identified.	Jain et al. 2014
11	Two Korean <i>japonica</i> rice varieties(Junam and Nampyeong).	352,478 SNPs and 45,645 InDels were identified between Junam and Nampyeong.	Jeong et al. 2015
12	Ten high yielding, Swarna, Samba Mahsuri, MTU1010, MTU1001, PKM-HMT, PR113, Pusa1121, Pooja, Satabdi and Sahabhagidhan.	Several SNPs and INDELs were identified. The number of SNPs and InDels varied from 23,23,105 (Samba Mahsuri) to 31,27,894 (Swarna) with an average of 27,52,180.	Behera et al. 2015

## Table 3. Examples of whole genome re-sequencing of rice genotypes.



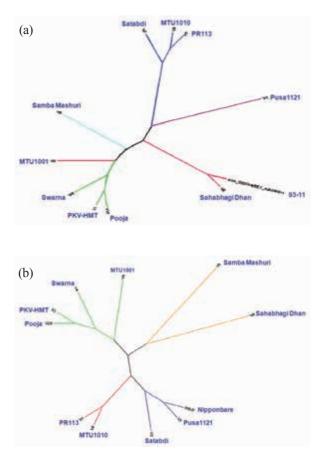
Sl No.	Genotypes Re-sequenced	DNA Polymorphism detected	References
13	Swarna	1,149,698 SNPs and 104,163 InDels were identified. 65,984 non- synonymous SNPs were identified in 20,350 genes. It helped to understand genetic basis of low glycemic index.	Rathinasa- bapathi et al. 2015
14	Kavuni, a traditional rice cultivar with nutritional and therapeutic properties.	1,150,711 SNPs were identified, of which 377,381 SNPs were located in the genic regions.	Rathinasa- bapathi et al. 2016
15	PDK Shriram.(high grain) and Heera (low grain).	2,337,784 and 2,471,741 SNPs and INDELs were identified in PDK Shriram and Heera, respectively. 1756 SNPs and 358 InDels were identified in PDK Shriram while 1941 SNPs and 412 InDels were identified in Heera among 41 yield trait specific genes.	Behera et al. 2016
16	Northern <i>japonica</i> rice variety Longdao24, and its parents Longdao5 and Jigeng83.	361,117 SNPs and 81,488 InDels were identified between Longdao24 and Longdao5 while 428,908 SNPs and 97,209 InDels were identified between Longdao24 genomeand Jigeng83.	Jiang et al. 2017
17	Salt tolerant rice cultivar SR86.	3,800,137 variants were identified, of which 85% were SNPs and 3.3% were INDELs.	Chen et al. 2017

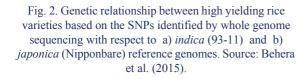
Behera et al. (2015) re-sequenced of ten high yielding mega rice varieties of India, namely, Swarna, Samba Mahsuri, MTU1010, MTU1001, PKM-HMT, PR113, Pusa1121, Pooja, Satabdi and Sahabhagidhan using NGS technology. They discovered a large number of DNA polymorphisms between these varieties, which would be useful for molecular breeding programs. Further, genetic relationship analysis indicated that Sahabhagidhan is more closer to *indica* reference genome, 93-11 while Pusa1121 is more closere to *japonica* reference genome, Nipponbare (Fig. 2). Behera et al. (2016) conducted whole genome re-sequencing of PDK Shriram (high grain) and Heera (low grain) rice cultivars and discovered a large number of DNA polymorphisms. The distribution pattern and annotation of SNPs and InDels were studied at 41 yield trait related genes. A total of 1756 SNPs and 358 InDels were identified in PDK Shriram while 1941 SNPs and 412 InDels were identified in Heera among 41 yield trait specific genes, which would be useful for basic and applied research.

# 6. KNOWLEDGE GAPS

Several rice cultivars have been sequenced and homology prediction identified to more than 54,000 genes in the genome. But, only few genes have been identified, well characterized and utilized in breeding programs for improving yield, grain quality, input use efficiency, tolerance to biotic and abiotic stresses, etc. It is highly necessary to identify more genes/QTLs from diverse genetic resources, characterize them,







understand functions. identify superior and beneficial alleles, and accumulate them in high vielding varieties for further improvement of vield, grain quality, input use efficiency, tolerance to biotic and abiotic stresses, and other desired traits. Still, a significant portion of beneficial/ superior alleles has not been discovered and used in the breeding programs. Salkathi is a highly BPH resistant land race and was used to introgress resistance into popular rice varieties Pusa44 and Samba Mahsuri. Two OTLS have been identified in Salkathi but mechanism of resistance of these OTLs is not known. Few OTLs for antioxidant properties and seedling vigor have been identified and few genes have been cloned. However, clear cut functions of those genes

Rice Research for Enhancing Productivity, Profitability and Climate Resilience

are still not known. Several high throughput genomic tools are available to address these problems.

The utilization of genome sequence information is meager though several rice genotypes have been sequenced, due to lack of strong bioinformatics expertise and facilities. Hence, human resource needs to be trained well to use NGS data efficiently. Very few high throughput facilities are available in India for precise phenotyping of germplasm for different traits and utilize them for allele mining, functional validation and finally use in breeding programs to develop high yielding, value aided and climate resilient rice varieties.



## 7. WAY FORWARD

Concerted efforts are needed for identification and fine mapping of genes/QTLs associated with traits like antioxidant properties, protein and micro nutrient contents of grains, seedling vigor and other agronomic traits, input use efficiency, resistance/ tolerance to biotic and abiotic stresses and use them in molecular breeding programs to increase value addition and climate resiliency in high yielding rice varieties. Further, emphasis should be given for discovery of superior alleles for different traits, and whole genome re-sequencing of key donors and elite cultivars has to be performed to identify SNPs, INDELs, understand the structure and functions of key genes related to yield traits, grain quality traits, tolerance to biotic and abiotic stresses, etc, and to develop markers for effective utilization in MAS programs for rice improvement.

## 8. SUMMARY

Improvement through conventional approaches has met with considerable success and rice production has doubled over the last 40 years. To feed growing population by the year 2030 AD, the global demand for rice would increase by requiring production of 858 million tons. Increasing rice yield to ensure food 40% challenge. Rice production is no longer keeping in pace with security is a major population growth during last decade because of Shrinking cultivable land area, water scarcity, depletion of soil fertility, globlal warming (climate change), evolution of new biotypes, pathotypes, etc. Hence, we have to use innovative tools to assist traditional methods in order to improve production and feed growing population. The availability of the complete genome sequence of rice and the developments in field of genomics has opened the door for speeding-up breeding processes for increasing yields and minimizing production risks. Genomics tools have been used for identification and mapping of genes/QTLs for target traits, whole genome sequencing of donors and elite genotypes for discovery of SNPs, genome-wide association mapping (GWAM), gene prospecting and allele mining, gene discovery for useful traits, precision breeding through MAS approach. Several QTLs/genes for biotic and abitic stresses, grain quality, early seedling vigor, input use efficiency, morphological and yield traits have been idenrtified. Few of them have been fine mapped, cloned and used in MAS breeding programs for developing climate regilient, input use efficient, nutrient rich and value aided high yielding rice varieties.

#### References

- Abdel-Aal ESM, Young JC and Rabalski I (2006) Anthocyanin composition in black, blue, pink, purple and red cereal grains. Journal of Agricultural and Food Chemistry 54: 4696-4704.
- Abe A, Takagi H, Fujibe T, Aya K, Kojima M, Sakakibara H, Uemura A, Matsuoka M and Terauchi R (2012) OsGA200x1, a candidate gene for a major QTL controlling seedling vigor in rice. Theoretical and Applied Genetics 125(4): 647-57.
- Abdurakhmonov IY and Abdukarimov A (2008) Application of Association Mapping for Understanding the Genetic Diversity of Plant Germplasm Resources. International Journal of Plant Genomics doi: 10.1155/2008/574927.



- Agrama HA, Eizenga CG and Yan W (2007) Association mapping of yield and its components in rice cultivars. Molecular Breeding 19:341-356.
- Al-Shugeairy Z, Price AH and Robinson D (2015) Genome-Wide Association Mapping for Drought Recovery Trait in Rice (*Oryza sativa* L.). International Journal of Applied Agricultural Sciences 1(1): 11-18.
- Ashikari M and Matsuoka M (2008) Identification, isolation and pyramiding of quantitative trait loci for rice breeding. Trends in Plant Science 11(7): 344-50.
- Anandan A, Anumalla M, Pradhan SK and Ali J (2016) Population structure, diversity and trait association analysis in rice (*Oryza sativa* L.) germplasm for Early Seedling Vigor (ESV) using trait linked SSR markers. PLoS ONE 11(3): e0152406.
- Arab F, Alemzadeh I and Maghsoudi V (2011) Determination of antioxidant component and activity of rice bran extract. Scientia Iranica 18 (6): 1402-1406.
- Arai-Kichise Y, Shiwa Y, Nagasaki H, Ebana K, Yoshikawa H, Yano M and Wakasa K (2011) Discovery of genome-wide DNA polymorphisms in a landrace cultivar of *japonica* rice by whole-genome sequencing. Plant and Cell Physiology 52: 274–282.
- Arai-Kichise Y, Shiwa Y, Ebana K, Shibata-Hatta M, Yoshikawa H,Yano M and Wakasa K (2014) Genome-wide DNA polymorphisms in seven rice cultivars of temperate and tropical *japonica* groups. PLoS ONE 9(1): 1-10.
- Behera L, Nanda A, Sahu RK, Jena M, Sahu SC, Patnaik A, Rao GJN and Singh ON (2012) DNA fingerprinting of brown planthopper resistant rice cultivars. Oryza 49(2):80-87.
- Behera L, Umakanta N, Ray S, Katara JL, Samantray S, Subudhi HN, Dash SK, Pradhan SK, Singh ON and Mohapatra M (2015) Discovery of genome-wide DNA polymorphisms in high yielding mega rice varieties of India using NGS technology. In: International Rice Symposium-IRS2015,18-20 November, Hyderabad, IRS15-OP448.
- Behera L, Mohanty S, Mohanty N, Gouda G, Pradhan SK, Dash SK, Ngangkham U, Jambhulkar NN and Singh ON (2016) Discovery of genome-wide DNA polymorphisms in high and low grain number rice cultivars using NGS technology. *In:* 2016 NextGen Genome, Biology, Bioinformatics and Technologies (NGBT) Conference, 3-5th October 2016 at Cochin, Kerala.
- Bentley DR (2006) Whole-genome re-sequencing. Current Opinion in Genetics and Development 16:545-552.
- Bimolata W, Kumar A, Sundaram RM, Laha GS, Qureshi IA and Ghazi IA (2015) Nucleotide diversity analysis of three major bacterial blight resistance genes in rice. PloS One 10(3): e0120186.
- Bohn L, Meyer AS and Rasmussen SK (2008) Phytate: impact on environment and human nutrition. A challenge for molecular breeding. Journal of Zhejiang University Science B 9(3):165–191.
- Buu BC, Ha PTT, Tam BP, Nhien TT, Hieu NV, Phuoc NT, Minh LT, Giang LH and Lang NT (2014) Quantitative trait loci associated with heat tolerance in rice (*Oryza sativa* L.) Plant Breeding and Biotechnolgy 2:14–24.
- Cao L, Zhao J, Zhan X, Li D, He L and Cheng S (2003) Mapping QTLs for heat tolerance and correlation between heat tolerance and photosynthetic rate in rice. Chinese Journal of Rice Science 17:223–227.
- Challam C, Ghosh T, Rai M and Tyagi W (2015) Allele mining across *DREB1A* and *DREB1B* in diverse rice genotypes suggest a highly conserved pathway inducible by low temperature. Journal of Genetics 94(2): 231-238.



- Chang-Lan Z, Ying-Hui X, Chun-Ming W, Ling J, Hu-Qu Z and Jian-Min W (2005) Mapping QTL for heat-tolerance at grain filling stage in rice. Rice Science 12(1):33–38.
- Chen Q, Yu S, Li C and Mou T (2008) Identification of QT s for heat tolerance at flowering stage in rice. Scientia Agricultura Sinica 41(2):315-321.
- Chen H, Xie W, He H, Yu H, Chen W, Li J, Yu R, Yao Y, Zhang W, He Y, Tang X, Zhou F, Deng XW and Zhang Q (2014) A high-density SNP genotyping array for rice biology and molecular breeding. Molecular Plant 7(3):541–553.
- Chen R, Cheng Y, Han S, Van Handel B, Dong L, Li X and Xie X (2017) Whole genome sequencing and comparative transcriptome analysis of a novel seawater adapted, salt-resistant rice cultivar - sea rice 86. BMC Genomics 18(1):655, doi: 10.1186/s12864-017-4037-3.
- Cheng L, Wang J, Uzokwe V, Meng L, Wang Y, Sun Y, Zhu L, Xu J and Li Z (2012) Genetic analysis of cold tolerance at seedling stage and heat tolerance at anthesis in rice (*Oryza sativa* L.). Journal of Integrative Agriculture 11:359–367.
- Cheng J, He Y, Yang B, Lai Y, Wang Z and Zhang H (2015) Association mapping of seed germination and seedling growth at three conditions in *indica* rice (*Oryza sativa* L.). Euphytica 206: 103-115.
- Cicero AFG and Gaddi A (2001) Rice bran oil and c-oryzanol in the treatment of hyperlipoproteinaemias and other conditions. Phototherapy Research 15:277–289.
- Courtois B, Audebert A, Dardou A, Roques S, Ghneim Herrera T, Droc G, Frouin J, Rouan L, Goze E, Kilian A, Ahmadi N and Dingkuhn M (2013) Genome-wide association mapping of root traits in a japonica rice panel. PLoS One 8(11): e78037.
- Dang X, Thi TG, Dong G, Wang H, Edzesi WM and Hong D (2014) Genetic Diversity and Association Mapping of Seed Vigor in Rice (*Oryza Sativa* L.). Planta 239: 1309–1319.
- Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM and Blaxter ML (2011) Genomewide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics 12:499–510.
- Deen R, Ramesh K, Gautam SK, Rao YK, Lakshmi VJ, Viraktamath BC, Brar DS and Ram T (2010) Identification of new gene for BPH resistance introgressed from *O. rufipogon*. Rice Genetics Newsletter 25:70–72.
- Deen R, Ramesh K, Padmavathi G, Viraktamath BC and Ram T (2017) Mapping of brown planthopper [*Nilaparvata lugens*(Sta°l)]. Euphytica 213: 35, DOI 10.1007/s10681-016-1786-z.
- Du B, Zhang WL, Liu BF, Hu J, Wei Z, Shi ZY, He RF, Zhu LL, Chen RZ and Han B (2009) Identification and characterization of *Bph14*, a gene conferring resistance to brown planthopper in rice. Proceedings of the National Academy of Sciences, USA 52: 22163-22168.
- Famoso AN, Zhao K, Clark RT, Tung CW, Wright MH, Bustamante C, Kochian LV and McCouch SR (2011) Genetic Architecture of Aluminum Tolerance in Rice (*Oryza sativa*) Determined through Genome-Wide Association Analysis and QTL Mapping. PLoS Genetics 7(8): e1002221.
- Fasahat F, Muhammad K, Abdullah A and Ratnam W (2012) Proximate nutritional composition and antioxidant properties of *Oryza rufipogon*, a wild rice collected from Malaysia compared to cultivated rice, MR219. Australian Journal of Crop Science 6(11):1502-1507.
- Fei-fei Xu, Liang JIN, Yan H, Chuan T, Ya-ling C and Jin-song B (2016) Association mapping of quantitative trait loci for yield-related agronomic traits in rice (*Oryza sativa* L.). Journal of Integrative Agriculture 15(10): 2192–2202.



- Feltus FA, Wan J, Schulze SR, Estill JC, Jiang N and Paterson AH (2004) An SNP resource for rice genetics and breeding based on subspecies *indica* and *japonica* genome alignments. Genome Research 14:1812–1819.
- Fujita D, KohliA and Horgan FG (2013) Rice resistance to planthoppers and leafhoppers. Critical Reviews in Plant Sciences 32(3):162-191.
- Furukawa T, Maekawa M, Oki T, Suda I, Iida S, Shimada H, Takamure I and Kadowaki KI (2007) The Rc and Rd genes are involved in proanthocyanidin synthesis in rice pericarp. The Plant Journal 49:91–102.
- Gao Q, Yue G, Li W, Wang J, Xu J and Yin Y (2012) Recent progress using high-throughput sequencing technologies in plant molecular breeding. Journal of Integrative Plant Biology 54:215–227.
- Gemede HM (2014) Potential health benefts and adverse eûects associated with phytate in foods. Food Science and Quality Management 27:45–54.
- Goff SA, Ricke D, Lan TH, Presting G, Wang R, Dunn M, Glazebrook J, Sessions A, Oeller P, Varma H, Hadley D, Hutchison D, Martin C, Katagiri F, Lange BM, Moughamer T, Xia Y, Budworth P, Zhong J, Miguel T, Paszkowski U, Zhang S, Colbert M, Sun WL, Chen L, Cooper B, Park S, Wood TC, Mao L, Quail P,Wing R, Dean R, Yu Y, Zharkikh A, Shen R, Sahasrabudhe S, Thomas A, Cannings R, Gutin A, Pruss D, Reid J, Tavtigian S, Mitchell J, Eldredge G, Scholl T,Miller RM, Bhatnagar S, Adey N, Rubano T, Tusneem N, Robinson R, Feldhaus J, Macalma T, Oliphant A and Briggs S (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp.*japonica*). Science 296: 92–100.
- Goffman FD and Bergman CJ (2004) Rice kernel phenolic content and its relationship with antiradical efficiency. Journal of The Science of Food and Agriculture 10:1002-1007.
- Goswami, S, Kar RK, Paul A and Dey N (2017) Genetic potentiality of indigenous rice genotypes from Eastern India with reference to submergence tolerance and deepwater traits. Current Plant Biology 11-12: 23-32.
- Gur A and Zamir D (2004) Unused natural variation can lift yield barriers in plant breeding. PLoS Biology 10(2): e245.
- Hall D, Tegstrom C and Ingvarsson PK (2010) Using association mapping to dissect the genetic basis of complex traits in plants. Briefings in Functional Genomics 9:157-165.
- Han B and Huang X (2013) Sequencing-based genome-wide association study in rice. Current Opinion in Plant Biology 16: 133-138.
- Hibino H (1996) Biology and epidemiology of rice viruses. Annual Review of Phytopathology 34: 249-274.
- Hu C, Zawistowski J, Ling W and Kitts DD (2003) Black rice (*Oryza sativa* L. *indica*) pigmented fraction suppresses both reactive oxygen species and nitric oxide in chemical and biological model systems. Journal of Agricultural and Food Chemistry 51:5271-5277.
- Hu J, Li X, Wu C, Yang C, Hua H, Gao G, Xiao J and He Y (2012) Pyramiding and evaluation of the brown planthopper resistance genes *Bph14* and *Bph15* in hybrid rice. Molecular Breeding 29:61-69.
- Hu J, Cheng M, Gao G, Zhang Q, Xiao J and He Y (2013) Pyramiding and evaluation of three dominant brown planthopper resistance genes in the elite *indica* rice 9311 and its hybrids. Pest Management Science 69: 802–808.
- Hu Y, Mao B, Peng Y, Sun Y, Pan Y, Xia Y, Li Y, Tang Li, Yuan L and Zhao B (2014) Deep resequencing of a widely used maintainer line of hybrid rice for discovery of DNA polymorphisms and evaluation of genetic diversity. Molecular and General Genetics 289(3): DOI 10.1007/s00438-013-0807-z.



- Hu J, Xiao C, Cheng M, Gao G, Zhang Q and He Y (2015) Fine mapping and pyramiding of brown planthopper resistance genes *QBph3* and *QBph4* in an introgression line from wild rice *O. officinalis.* Molecular Breeding 35:3, doi: 10.1007/s11032-015-0228-2.
- Hu J, Xiao C and He Y (2016) Recent progress on the genetics and molecular breeding of brown planthopper resistance in rice. Rice 9:30, doi: 10.1186/s12284-016-0099-0.
- Huang X, Wei X, Sang T, Zhao Q, Feng Q, Zhao Y, Li C, Zhu C, Lu T, Zhang Z, Li M, Fan D, Guo Y, Wang A, Wang L, Deng L, Li W, Lu Y, Weng Q, Liu K, Huang T, Zhou T, Jing Y, Li W, Lin Z, Buckler ES, Qian Q, Zhang QF, Li J and Han B (2010) Genome-wide association studies of 14 agronomic traits in rice landraces. Nature Genetics 42(1): 961-967.
- Huang Y, Sun C, Min J, Chen Y, Tong C and Bao J (2015) Association mapping of quantitative trait loci for mineral element contents in whole grain rice (*Oryza sativa* L.). Journal of Agricultural and Food Chemistry 63: 10885–10892.
- Hudson EA, Dinh PA, Kokubun T, Simmonds MSJ and Gescher A (2000) Characterization of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. Cancer Epidemiology, Biomarkers and Prevention 9:1163-1170.
- Imam J, Mandal NP, Variar M and Shukla P (2016) Allele mining and selective patterns of *Pi9* gene in a set of rice landraces from India. Frontiers in Plant Science 7: 1846, doi: 10.3389/ fpls.2016.01846.
- Jagadish SVK, Craufurd PQ and Wheeler TR (2008) Phenotyping parents of mapping populations of rice (*Oryza sativa* L.) for heat tolerance during anthesis. Crop Science 48:1140-1146.
- Jagadish SVK, Cairns J, Lafitte R, Wheeler TR, Price AH and Craufurd PQ (2010) Genetic analysis of heat tolerance at anthesis in rice. Crop Science 50:1633-1641.
- Jain M, Moharana KC, Shankar R, Kumari R and Garg R (2014) Genome-wide discovery of DNA polymorphisms in rice cultivars with contrasting drought and salinity stress response and their functional relevance. Plant Biotechnology Journal 12: 253–264.
- Jairin J, Teangdeerith S, Leelagud P, Kothcharerk J, Sansen K, Yi M, Vanavichit A and Toojinda T (2009) Development of rice introgression lines with brown planthopper resistance and KDML105 grain quality characteristics through marker assisted selection. Field Crops Research 110: 263-271.
- Jena KK, Pasalu IC, Rao YK, Varalaxmi Y, Krishnaiah K, Khush GS and Kochert G (2002) Molecular tagging of a gene for resistance to brown planthopper in rice (*Oryza sativa* L.). Euphytica 129: 81-88.
- Jena KK and SM Kim (2010) Current status of brown plant hopper (BPH) resistance and genetics. Rice 3: 161-171.
- Jeong IS, Kim TH, Lee SB, Suh SC and Ji H (2015) Genome-wide Detection of DNA polymorphisms between two Korean *japonica* rice varieties. Plant Breeding and Biotechnology 3(3):208-215.
- Ji H, Kim SR, Kim YH, Suh JP, Park HM, Sreenivasulu N, Misra G, Kim SM, Hechanova SL, Kim H, Lee GS, Yoon UH, Kim TH, Lim H, Suh SC, Yang J, An G and Jena KK (2016) Mapbased cloning and characterization of the *BPH18* gene from wild rice conferring resistance to brown plant hopper (BPH) Insect Pest. Scientific Reports 6:34376, doi: 10.1038/ srep34376.
- Jiang S, Sun S, Bai L, Ding G, Wang T, Xia T, Jiang H, Zhang X and Zhang F (2017) Resequencing and variation identification of whole genome of the japonica rice variety "Longdao24" with high yield. PLoS One 12(7): e0181037.
- Jin L, Xiao P, Lu Y, Shao YF, Shen Y and Bao JS (2009) Quantitative trait loci for brown rice color, total phenolics and, flavonoid contents and antioxidant capacity in rice grain. Cereal Chemistry 86:609-615.



- Jin L, Lu Y, Xiao P, Sun M, Corke H and Bao J (2010) Genetic diversity and population structure of a diverse set of rice germplasm for association mapping. Theoretical and Applied Genetics 121: 475–487.
- Kim SR, Ramos J, Ashikari M, Virk PS, Torres EA, Nissila E and Jena KK (2016) Development and validation of allele-specific SNP/indel markers for eight yield-enhancing genes using wholegenome sequencing strategy to increase yield potential of rice, *Oryza sativa* L. Rice 9(1): 12, DOI 10.1186/s12284-016-0084-7.
- Kumar V, Singh A, Mithra SVA, Krishnamurthy SL, Parida SK, Jain S, KK, Kumar P, Rao AR, Sharma SK, Khurana JP, Singh NK and Mohapatra T (2015) Genome-wide association mapping of salinity tolerance in rice (*Oryza sativa*). DNA Research 22: 133-145.
- Kovach MJ and McCouch SR (2008) Leveraging natural diversity: back through the bottleneck. Current Opinion in Plant Biology 11: 193-200.
- Kumari S, Jennifer MS, Maheshwaran M, Shanmugasunderam P, Suresh S, Pothi N, Subbarayalu M, Muthurajan R and Natesan S (2010) Screening of IR50 3 Rathu Heenati F7 RILs and Identification of SSR Markers Linked to Brown Planthopper (Nilaparvata lugens StaÚl) Resistance in Rice (*Oryza sativa* L.). Molecular Biotechnology 46: 63-71.
- Li X, Yan X, Agrama HA, Jia L, Jackson A, Moldenhauer K, Yeater K, McClung A and Wu D (2012) Unraveling the Complex Trait of Harvest Index with Association Mapping in Rice (*Oryza sativa* L.). PLoS ONE 7(1): e29350.
- Li S, Wang S, Deng Q, Zheng A, Zhu J, Liu H, Wang L, Gao F, Zou T, Huang B, Cao X, Xu L, Yu C, Ai P and Li P (2012b) Identification of genome-wide variations among three elite restorer lines for hybrid-rice. PLoS One 7: e30952.
- Li JY, Wang J and Zeigler RS (2014) The 3000 Rice Genome Project: new opportunities and challenges for future rice research. GigaScience 3: 8, doi: 10.1186/2047-217X-3-8.
- Li XM, Chao DY, Wu Y, Huang X, Chen K, Cui LG and Dong NQ (2015) Natural alleles of a proteasome [alpha] 2 subunit gene contribute to thermotolerance and adaptation of African rice. Nature Genetics 47(7): 827-833.
- Ling KC, Tiongco ER and Aguiero VM (1978) Rice ragged stunt, a new virus disease. The Plant Disease Reporter 62: 701-705.
- Ling WH, Cheng QX, Ma J and Wang T (2001) Red and black rice decrease atherosclerotic plaque formation and increase antioxidant status in rabbits. The Journal of Nutrition 131:1421-1426.
- Liu RH (2007) Whole grain phytochemicals and health. Journal of Cereal Science 46:207–219.
- Liu Y, Wu H, Chen H, Liu Y, He J, Kang H, Sun Z, Pan G, Wang Qi, Hu J, Zhou F, Zhou K, Zheng X, Ren Y, Chen L, Wang Y, Zhao Z, Lin Q, Wu F, Zhang X, Guo X, Cheng X, Jiang L, Wu C, Wang H and Wan J (2015) A gene cluster encoding lectin receptor kinases confers broad-spectrum and durable insect resistance in rice. Nature Biotechnology 33(3): 301-305.
- Lou Q, Chen L, Mei H, Wei H, Feng F, Wang P, Xia H, Li T and Luo L (2015) Quantitative trait locus mapping of deep rooting by linkage and association analysis in rice. Journal of Experimental Botany 66(15): 4749-4757.
- Lu, Q, Zhang M, Niu X, Wang S, Xu Q, Feng Y, Wang C, Deng H, Yuan X, Yu H, Wang Y and Wei X (2015) Genetic Variation and Association Mapping for 12 Agronomic Traits in *Indica* Rice. BMC Genomics 16: 1067, doi.org/10.1186/s12864-015-2245-2.
- Lu Q, Zhang M, Niu X, Wang C, Xu Q, Feng Y, Wang S, Yuan X, Yu H, Wang Y and Wei X (2016) Uncovering novel loci for mesocotyl elongation and shoot length in *indica* rice through genome-wide association mapping. Planta 243: 645-657.



- Lv Q, Huang Z, Xu X, Tang L, Liu H, Wang C and Li X (2017) Allelic variation of the rice blast resistance gene Pid3 in cultivated rice worldwide. Scientific Reports 7(1): 10362, DOI:10.1038/s41598-017-10617-2.
- Ma X, Feng F, Wei H, Mei H, Xu K, Chen S, Li T, Liang X, Liu H and Luo L (2016) Genome-Wide Association Study for Plant Height and Grain Yield in Rice under Contrasting Moisture Regimes. Frontiers in Plant Science 7:1801, doi: 10.3389/fpls.2016.01801.
- Maeda H, Yamaguchi T, Omoteno M, Takarada T, Fujita K, Murata K, Iyama Y, Kojima Y, Morikawa M, Ozaki H, Mukaino N, Kidani Y and Ebitani T (2014) Genetic dissection of black grain rice by the development of a near isogenic line. Breeding Science 64: 134-141.
- Mahender A, Anandan A and Pradhan SK (2015) Early seedling vigor, an imperative trait for direct seeded rice: an overview on physio-morphological parameters and molecular markers. Planta 241: 1027-1050.
- McCouch SR, Zhao K, Wright M, Tung CW, Ebana K, Thomson M, Reynolds A, Wang D, DeClerck G, Ali MdL, McClung A, Eizenga G and Bustamante C (2010) Development of genome-wide SNP assays for rice. Breeding Science 60:524–535.
- McNally KL, Childs KL, Bohnert R, Davidson RM, Zhao K, Ulat VJ, Zeller G, Clark RM, Hoen DR, Bureau TE, Stokowski R, Ballinger DG, Frazer KA, Cox DR, Padhukasahasram B, Bustamante CD, Weigel D, Mackill DJ, Bruskiewich RM, Rätsch G, Buell CR, Leung H and Leach JE (2009) Genome-wide SNP variation reveals relationships among landraces and modern varieties of rice. Proceedings of the National Academy of Sciences, USA 106(30):12273-2278.
- Mohanty SK, Panda RS, Mohapatra SL, Nanda A, Behera L, Jena M, Sahu RK, Sahu SC and Mohapatra T (2017) Identification of novel quantitative trait loci associated with brown planthopper resistance in the rice landrace Salkathi. Euphytica 213: 38.
- Muthukumar C, Subathra T, Aiswarya J, Gayathri V and Chandra Babu R (2015) Comparative genome-wide association studies for plant production traits under drought in diverse rice (Oryza sativa L.) lines using SNP and SSR markers. Current Science 109:139-47.
- Nagavarapu T, Kunusooth K , Subba Rao LV, Senguttuvel P and Maganti SM (2017) Validation of Seedling Vigour QTLs in Rice (*Oryza sativa* L.). Current Trends in Biotechnology and Pharmacy 11 (1): 24-33.
- Neelam K, Thakur S, Neha, Yadav IS, Kumar K, Dhaliwal SS, and Singh K (2017) Novel alleles of Phosphorus-Starvation Tolerance 1 gene (*PSTOL1*) from Oryza rufipogon confers high phosphorus uptake efficiency. Frontiers in Plant Science 8: 508, doi: 10.3389/ fpls.2017.00509.
- Padmavathi G, Ram T, Pasalu IC and Mishra B (2005) Gene identification of brown planthopper (*Nilaparvata lugens*) resistance in rice (*Oryza sativa*). Indian Journal of Agricultural Sciences 75(9):587-590.
- Pan Y, Zhang H, Zhang D, Li J, Xiong H, Yu J, Li J, Rashid MAR, Li G, Ma X, Cao G, Han L and Li Z (2015) Genetic analysis of cold tolerance at the germination and booting stages in rice by association mapping. PLoS ONE 10: e0120590.
- Pandit E, Tasleem S, Barik SR, Mohanty DP, Nayak DK, Mohanty SP, Das S and Pradhan SK (2017) Genome-Wide Association Mapping Reveals Multiple QTLs Governing Tolerance Response for Seedling Stage Chilling Stress in Indica Rice. Frontiers in Plant Science 8: 552, doi: 10.3389/fpls.2017.0055.
- Phung NTF, Mai CD, Hoang GT, Truong HTM, Lavarenne J, Gonin M, Nguyen KL, Ha TT, Do VN, Gantet P and Courtois B (2016) Genome-wide association mapping for root traits in a panel of rice accessions from Vietnam. BMC Plant Biology 16: 64, doi.org/10.1186/ s12870-016-0747-y.



- Platten JD, Egdane JA and Ismail AM (2013) Salinity tolerance, Na+ exclusion and allele mining of HKT1; 5 in Oryza sativa and O. glaberrima: many sources, many genes, one mechanism?. BMC Plant Biology 13(1): 32, doi: 10.1186/1471-2229-13-32.
- Poli Y, Basava RK, Panigrahy M, Vinukonda VP, Dokula NR, Voleti SR, Desiraju S and Neelamraju S (2013) Characterization of a Nagina22 rice mutant for heat tolerance and mapping for yield traits. Rice 6: 36, doi.org/10.1186/1939-8433-6-36.
- Prahalada GD, Shivakumar N, Lohithaswa HC, Sidde Gowda DK, Ramkumar G, Kim SR, Ramachandra C, Hittalmani S, Mohapatra T and Jena KK (2017) Identification and fine mapping of a new gene, *BPH31* conferring resistance to brown planthopper biotype 4 of India to improve rice, *Oryza sativa* L. Rice 10: 41, DOI 10.1186/s12284-017-0178-x.
- Pradhan SK, Barik SR, Sahoo A, Mohapatra S, Nayak DK, Mahender A, Meher J, Anandan A and Pandit E (2016) Population structure, genetic diversity and molecular marker-trait association analysis for high temperature stress tolerance in rice. PLoS ONE 11: e0160027.
- Prasad PVV, Boote KJ, Jr LHA, Sheehy JE and Thomas JMG (2006) Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. Field Crops Research 95:398–411.
- Prasanth VV, Basava KR, Babu MS, Tripura Venkata VGN, Devi SJ, Mangrauthia SK, Voleti SR and Sarla N (2016) Field level evaluation of rice introgression lines for heat tolerance and validation of markers linked to spikelet fertility. Physiology and Molecular Biology of Plants 22: 179-182.
- Rathinasabapathi P, Purushothaman N, Ramprasad VL and Parani M (2015) Whole genome sequencing and analysis of Swarna, a widely cultivated *indica* rice variety with low glycemic index. Scientific Reports 5:11303, DOi: 10.1038/srep11303.
- Rathinasabapathi P, Purushothaman N and Parani M (2016) Genome-wide DNA polymorphisms in Kavuni, a traditional rice cultivar with nutritional and therapeutic properties. Genome 59(5): 363-366.
- Rebolledo MC, Peña AL, Duitama J, Cruz DF, Dingkuhn M, Grenier C and Tohme J (2016) Combining Image Analysis, Genome Wide Association Studies and Different Field Trials to Reveal Stable Genetic Regions Related to Panicle Architecture and the Number of Spikelets per Panicle in Rice. Frontiers in Plant Science 7: 1384, doi: 10.3389/ fpls.2016.01384.
- Reddy VS, Dash S and Reddy AR (1995) Anthocyanin pathway in rice (*Oryza sativa* L.): identification of a mutant showing dominant inhibition of anthocyanins in leaf and accumulation of proanthocyanidins in pericarp. Theoretical and Applied Genetics 91: 301-312.
- Ren J, Gao F, Wu X, Zeng L, Lv J, Su X, Luo H and Ren G (2016) BPH32, a novel gene encoding an unknown SCR domain-containing protein, confers resistance against the brown planthopper in rice. Scientific Reports 6: 37645.
- Rivera CT, Ou SH and Lida TT (1996) Grassy stunt disease of rice and its transmission by *Nilaparvata lugens(stal.)*. The Plant Disease Reporter 50:453-456.
- Sanghamitra P, Baran Bagchi TB, Sah RP, Sharma SG, Sarkar S and Basak N (2017) Characterization of red and purple-pericarp rice (*Oryza sativa* L.) based on physico-chemical and antioxidative properties of grains. Oryza 54: 57-64.
- Shao Y, Jin L, Zhang G, Lu Y, Shen Y and Bao J (2011) Association mapping of grain color, phenolic content, flavonoid content and antioxidant capacity in dehulled rice. Theoretical and Applied Genetics 122:1005–1016.
- Shanmugavadivel PS, Amitha Mithra SV, Prakash C, Ramkumar MK, Ratan T, Mohapatra T and Singh NK (2017) High Resolution Mapping of QTLs for Heat Tolerance in Rice Using a 5K SNP Array. Rice 10(1): 28, doi: 10.1186/s12284-017-0167-0.



- Sharma PN, Torii A, Takumi S, Mori N and Nakamura C (2004) Marker-assisted pyramiding of brown planthopper (*Nilaparvata lugens* Stal) resistance genes *Bph1* and *Bph2* on rice chromosome 12. Hereditas 140: 61-69.
- Shen Y, Jin L, Xiao P, Lu Y and Bao JS (2009) Total phenolics, flavonoids, antioxidant capacity in rice grain and their relations to grain color, size and weight. Journal of Cereal Science 49:106–111.
- Shen Y, Wan Z, Coarfa C, Drabek R, Chen L, Ostrowski EA, Liu Y, Weinstock GM, Wheeler DA, Gibbs RA and Yu F (2010) A SNP discovery method to assess variant allele probability from next-generation resequencing data. Genome Research 20(2): 273-280.
- Singh UM, Yadav S, Dixit S, Ramayya PJ, Devi MN, Raman KA and Kumar A (2017) QTL Hotspots for Early Vigor and Related Traits under Dry Direct-Seeded System in Rice (*Oryza sativa* L.). Frontiers in Plant Science 8: 286, doi: 10.3389/fpls.2017.00286.
- Sogawa K (1982) The rice brown planthopper: feeding physiology and host plant interactions. Annual Review of Entomology 27: 49-73.
- Soundararajan RP, Kadirvel P, Gunathilagaraj K and Maheswaran M (2004) Mapping of Quantitative Trait Loci Associated with Resistance to Brown Planthopper in Rice by Means of a Doubled Haploid Population. Crop Science 44:2214–2220.
- Stich B and Melchinger AE (2010) An introduction to association mapping in plants. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 5: 039, doi: 10.1079/PAVSNNR20105039.
- Subbaiyan GK, Waters DL, Katiyar SK, Sadananda AR, Vaddadi S and Henry RJ (2012) Genomewide DNA polymorphisms in elite indica rice inbreds discovered by whole-genome sequencing. Plant Biotechnology Journal 10: 623–634.
- Suh JP, Yang SJ, Jeung JU, Pamplona A, Kim JJ, Lee JH, Hong HC, Yang CI, Kim YG and Jena KK (2011) Development of elite breeding lines conferring *Bph18* gene-derived resistance to brown planthopper (BPH) by marker-assisted selection and genome-wide background analysis in Japonica rice (*Oryza sativa* L.). Field Crops Research 120:68-75.
- Swamy BPM, Shamsudin NAZ, Rahman SNA, Mauleon R, Ratnam W, Cruz MTS and Kumar A (2017) Association Mapping of Yield and Yield related Traits Under Reproductive Stage Drought Stress in Rice (*Oryza sativa* L.). Rice 10: 21, doi: 10.1186/s12284-017-0161-6.
- Sweeney MT, Thomson MJ, Pfeil BE and McCouch SR (2006) Caught red-handed: Rc encodes a basic helix–loop–helix protein conditioning red pericarp in rice. Plant Cell 18: 283-294.
- Tamura Y, Hattori M, Yoshioka H, Yoshioka M, Takahashi A, Wu J, Sentoku N and Yasui H (2014) Map-based cloning and characterization of a brown planthopper resistance gene *BPH26* from *Oryzasativa* L. ssp. *indica* cultivar ADR52. Scientific Reports 4: 5872, doi: 10.1038/srep05872.
- Tan YF, Sun M, Xing YZ, Hua JP, Xun XL, Zhang QF and Corke H (2001) Mapping quantitative trait loci for milling quality, protein content and color characteristics of rice using a recombinant inbred line population derived from an elite rice hybrid. Theoretical and Applied Genetics 103:1037–1045.
- Tazib T, Kobayashi Y, Koyama H and Matsui T (2015) QTL analyses for anther length and dehiscence at flowering as traits for the tolerance of extreme temperatures in rice (*Oryza* sativa L.) Euphytica 203:629–642.
- Teixeira EI, Fischer G, van Velthuizen H, Walter C and Ewert F (2013) Global hot-spots of heat stress on agricultural crops due to climate change. Agricultural and Forest Meteorology 170: 206-215.



- Tian S, Nakamura K and Kayara H (2004) Analysis of phenolic compounds in white rice, brown rice and germinated brown rice. Journal of Agricultural and Food Chemistry 52:4808–4813.
- Varshney RK, Nayak SN, May GD and Jackson SA (2009) Next-generation sequencing technologies and their implications for crop genetics and breeding. Trends in Biotechnology 27:522-530.
- Vasudevan K, Gruissem W and Bhullar NK (2015) Identification of novel alleles of the rice blast resistance gene Pi54. Scientific Reports 5: 15678, doi:10.1038/srep15678.
- Wang CX and Shu QY (2007) Fine mapping and candidate gene analysis of purple pericarp gene Pb in rice (*Oryza sativa* L.). Chinese Science Bulletin 52:3097–3104.
- Wang Y, Cao L, Zhang Y, Cao C, Liu F, Huang F, Qiu Y, Li R and Lou X (2015a) Map-based cloning and characterization of *BPH29*, a B3 domain-containing recessive gene conferring brown planthopper resistance in rice. Journal of Experimental Botany 66: 6035-6045.
- Wang Q, Xie W, Xing H, Yan J, Meng X, Li X, Fu X, Xu J, Lian X, Yu S, Xing Y and Wang G (2015b) Genetic architecture of natural variation in rice chlorophyll content revealed by a genome-wide association study. Molecular Plant 8: 946-957.
- Watanabe T and Kitagawa H (2000) Photosynthesis and translocation of assimilates in rice plants following phloem feeding by the planthopper *Nilaparvata lugens* (Homoptera: Delphacidae). Jornal of Economic Entomology 93:1192-1198.
- Walter ME, Marchesan PFS, Massoni, LP, da Silva GMS and Sartori RB Ferreira (2013) Antioxidant properties of rice grains with light brown, red and black pericarp colors and the effect of processing. Food Research International 50: 698–703.
- Welch JR, Vincentb JR, Auffhammer M, Moyae PF, Dobermann A and Daweg D (2010) Rice yields in tropical/subtropical Asia exhibit large but opposing sensitivities to minimum and maximum temperatures. Proceedings of the National Academy of Sciences, USA 107 (33): 14562-14567.
- Wu H, Liu Y, He J, Liu Yanling, Jiang L, Liu L, Wang C, Cheng X and Wan J (2014) Fine mapping of brown planthopper (*Nilaparvata lugens* Stàl) resistance gene *Bph28(t)* in rice (*Oryza sativa* L.). Molecular Breeding 33: 909-918.
- Wu J, Feng F, Lian X, Teng X, Wei H, Yu H, Xie W, Yan M, Fan P, Li Y, Ma X, Liu H, Yu S, Wang G, Zhou F, Luo L and Mei H (2015) Genome-wide Association Study (GWAS) of mesocotyl elongation based on re-sequencing approach in rice. BMC Plant Biology 15: 218, doi: 10.1186/s12870-015-0608-0.
- Xia M, Ling WH, Ma J, Kitts DD and Zawistowski J (2003) Supplementation of diets with the black rice pigment fraction attenuates atherosclerotic plaque formation in apolipoprotein E deficient mice. The Journal of Nutrition 133:744–751.
- Xiao YH, Pan Y, Luo L, Zhang G, Deng H, Dai L, Liu X, Tang W, Chen L and Wang GL (2011a) Quantitative trait loci associated with seed set under high temperature stress at the flowering stage in rice (*Oryza sativa* L.). Euphytica 178:331–338.
- Xiao YH, Pan Y, Luo LH, Deng HB, Zhang GL, Tang WB and Chen LY (2011b) Quantitative trait loci associated with pollen fertility under high temperature stress at flowering stage in rice (*Oryza sativa* L). Rice Science 18(2):1–7.
- Xiao Cong, Hu Jie, Ao Yi-Ting, Cheng Ming-Xing, Gao Guan-Jun, Zhang Qing-Lu, He Guang-Cun and He Yu-Qing (2016) Development and evaluation of near-isogenic lines for brown planthopper resistance in rice cv. 9311. Scientific Reports 6: 38159, DOI: 10.1038/ srep38159.



- Xu X, Liu X, Ge S, Jensen JD, Hu F, Li X, Dong Y, Gutenkunst RN, Fang L, Huang L, Li J, He W, Zhang G, Zheng X, Zhang F, Li Y,Yu C, Kristiansen K, Zhang X, Wang J, Wright M, McCouch SR, Nielsen R, Wang J and Wang W (2012) Resequencing 50 accessions of cultivated and wild rice yields markers for identifying agronomically important genes. Nature Biotechnology 30: 105-111.
- Xu F, Bao J, Kim TS and Park YJ (2016) Genome-wide Association Mapping of Polyphenol Contents and Antioxidant Capacity in Whole-Grain of Rice. Agricultural and Food Chemistry 64 (22):4695–4703.
- Xu TY, Sun J, Chang HL, Zheng HL, Wang JG, Liu HL, Yang LM, Zhao HW and Zou DT (2017) QTL mapping for anthocyanin and proanthocyanidin content in red rice. Euphytica 213: 243.
- Yamamoto T, Nagasaki H, Yonemaru JI, Ebana K, Nakajima M, Shibaya T and Yano M (2010) Fine definition of the pedigree haplotypes of closely related rice cultivars by means of genome-wide discovery of single-nucleotide polymorphisms. BMC Genomics 11:267, doi.org/10.1186/1471-2164-11-267.
- Yan WG, Li Y, Agrama HA, Luo D, Gao F, Lu X and Ren G (2009) Association mapping of stigma and spikelet characteristics in rice (*Oryza sativa* L.). Molecular Breeding 24: 277–292.
- Yang W, Guo Z, Huang C, Wang K, Jiang N, Feng H, Chen G, Liu Q and Xiong L (2015) Genomewide association study of rice (*Oryza sativa* L.) leaf traits with a high-throughput leaf scorer. Journal of Experimental Botany 66: 5605–5615.
- Yang X, Xia X, Zhang Z, Nong B, Zeng Y, Xiong F, Wu Y, Gao J, Deng G and Li D (2017) QTL Mapping by Whole Genome Re-sequencing and Analysis of Candidate Genes for Nitrogen Use Efficiency in Rice. Frontiers in Plant Science 8: 1634, doi: 10.3389/fpls.2017.01634.
- Yano K, Yamamoto E, Aya K, Takeuchi H, Lo PC, Hu L, Yamasaki M, Yoshida S, Kitano H, Hirano K and Matsuoka M (2016) Genome-wide association study using whole-genome sequencing rapidly identifies new genes influencing agronomic traits in rice. Nature Genetics 48: 927-934.
- Yawadio R, Tanimori S and Morita N (2007) Identification of phenolic compounds isolated from pigmented rices and their aldose reductase inhibitory activities. Food Chemistry 101:1644–1653.
- Ye C, Argayoso MA, Redoña ED, Sierra SL, Laza MA, Dilla CJ, Mo Y, Thomson MJ, Chin J, Delaviña CB, Diaz DQ and Hernandez JE (2012) Mapping QTL for heat tolerance at flowering stage in rice using SNP markers. Plant Breeding 131(1):33–41.
- Ye C, Tenorio FA, Argayoso MA, Laza MA, Koh HJ, Redoña ED, Jagadish KS and Gregorio GB (2015) Identifying and confirming quantitative trait loci associated with heat tolerance at flowering stage in different rice populations. BMC Genetics 16:41, doi.org/10.1186/s12863-015-0199-7.
- Yoshimura A, Ideta O and Iwata N (1997) Linkage map of phenotype and RFLP markers in rice. Plant Mol Biol 35:49-60.
- Yu J, Hu S, Wang J, Wong GK, Li S, Liu B, Deng Y, Dai L, Zhou Y, Zhang X, Cao M, Liu J, Sun J, Tang J, Chen Y, Huang X, Lin W, Ye C, Tong W, Cong L,Geng J, Han Y, Li L, Li W, Hu G, Huang X, Li W, Li J, Liu Z, Li L, Liu J, Qi Q, Liu J, Li L, Li T, Wang X, Lu H, Wu T, Zhu M, Ni P, Han H, Dong W, Ren X, Feng X,Cui P, Li X, Wang H, Xu X, Zhai W, Xu Z, Zhang J, He S, Zhang J, Xu J, Zhang K, Zheng X, Dong J, Zeng W, Tao L, Ye J, Tan J, Ren X, Chen X, He J, Liu D,Tian W, Tian C, Xia H, Bao Q, Li G, Gao H, Cao T, Wang J, Zhao W, Li P, Chen W, Wang X, Zhang Y, Hu J, Wang J, Liu S, Yang J, Zhang G, Xiong Y, Li Z, Mao L, Zhou C, Zhu Z, Chen R, Hao B, Zheng W, Chen S, Guo W, Li G, Liu S, Tao M, Wang J, Zhu L, Yuan Land Yang H (2002) A draft sequence of the rice genome (*Oryza sativa* L. ssp. *indica*). Science 296: 79–92.



- Yu P, Wang XM, Yuan XP, Wang CH, Xu Q, Feng Y and Wei XH (2016) Sequence variations and haplotypes of the bacterial blight resistance gene *xa13* in rice. Journal of Plant Pathology 98(1): 167-169.
- Zhang T, Yang L, Jiang K, Huang M, Sun Q, Chen W and Zheng J (2008) QTL mapping for heat tolerance of the tassel period of rice. Molecular Plant Breeding 6:867-873.
- Zhang G, Chen L, Xiao G, Xiao Y, Chen X and Zhang S (2009) Bulked segregant analysis to detect QTL related to heat tolerance in rice using SSR markers. Agricultural Sciences in China 8:482–487.
- Zhang J, Zhou X, Yan W, Zhang Z, Lu L, Han Z and Shen G (2015) Combinations of the *Ghd7*, *Ghd8* and *Hd1* genes largely define the ecogeographical adaptation and yield potential of cultivated rice. New Phytologist 208(4): 1056-1066.
- Zhang Y, Zhou L, Shen X, Chen D, Wu W, Zhan X, Liu Q, Zhu A, Lou X, Xu H, Cheng S and Cao L (2017) Genetic dissection of yield traits in super hybrid rice Xieyou9308 using both unconditional and conditional genome-wide association mapping. Scientific Reports 7: 824, DOI:10.1038/s41598-017-00938-7.
- Zhao K, Tung CW, Eizenga GC, Wright MH, Ali ML, Price AH, Norton GJ, Islam MR, Reynolds A, Mezey J, McClung AM, Bustamante CD and McCouch SR (2011) Genome-wide association mapping reveals a rich genetic architecture of complex traits in *Oryza sativa*. Nature Communications 2: 467, doi:10.1038/ncomms1467.
- Zhao M, Sun J, Xiao Z, Cheng F, Xu H, Tang L and Xu Q (2016a) Variations in DENSE AND ERECT PANICLE 1 (DEP1) contribute to the diversity of the panicle trait in highyielding japonica rice varieties in northern China. Breeding Science 66(4): 599-605.
- Zhao Y, Huang J, Wang Z, Jing S, Wang Y, Ouyang Y, Cai B, Xin XF, Liu X, Zhang C, Pan Y, Ma R, Li Q, Jiang W, Zeng Y, Shangguan X, Wang H, Du B, Zhu L, Xu X, Feng Yu-Qi, He SY, Chen R, Zhang Q and He G (2016b) Allelic diversity in an NLR gene *BPH9* enables rice to combat planthopper variation. Proceedings of the National Academy of Sciences, USA 113(45): 12850–12855.
- Zhao L, Lei J, Huang Y, Zhu S, Chen H, Huang R, Peng Z, Tu Q, Shen X and Yan S (2016c) Mapping quantitative trait loci for heat tolerance at anthesis in rice using chromosomal segment substitution lines. Breeding Science 66(3): 358–366.
- Zhou J, You A, Ma Z, Zhu L and He G (2012) Association analysis of important agronomic traits in *japonica* rice germplasm. African Journal of Biotechnology 11(12): 2957-2970.\*



# Nutrient Management for Enhancing Productivity and Nutrient Use Efficiency in Rice

## AK Nayak, S Mohanty, D Chatterjee, D Bhaduri, R Khanam, M Shahid, R Tripathi, A Kumar, S Munda, U Kumar, P Bhattacharyya, BB Panda and H Pathak

## SUMMARY

Development of appropriate management strategy for enhancing nutrient use efficiency, and ensuring environmental sustainability of rice production system is a priority area of research. Considerable progress has been made so far from broad based blanket nutrient recommendation to supply and demand based site specific nutrient recommendation. The nutrient management researches in rice till date mostly focus on "4 R" stewardship i.e. right dose, right time, right source and right place of nutrient application. Numerous technologies, tools and products such as soil test crop response (STCR) based N, P, K recommendation, optical sensor based real time N management, enhanced efficiency fertilizer materials have been developed and evaluated in rice and rice based systems to ensure 4 "R" principles of nutrient application and enhance yield and nutrient use efficiency. Further research is needed to fine tune these technologies for its wider adaptability and also to redefine nutrient management strategy in the context of climate change, next generation super, high protein and abiotic stress tolerant rice. It is essential to devise ecological intensification based nutrient recommendation that takes in to account ecological processes as well as the interactions among themselves to reduce negative environmental impact of chemical fertilizers.

## **1. INTRODUCTION**

Rice is one of the input intensive crops in the world and input of nutrient contributes approximately 20–25% to the total production costs of rice. At present rice production alone consumes nearly 24.7 Mt of fertilizer (N +  $P_2O_5 + K_2O$ ) which accounts for approximately 14.0% of total global fertilizer consumption in a year. Scientists have predicted that a hike of at least 60% in rice yield is essential in order to ensure food and nutritional security of 9 billion populations that are expected to inhabit the globe by 2050. With increasing demand for food production, demand for nutrients is likely to increase further.

Despite several decades of research the average recovery efficiency of N, P and K in rice is only 30-35%, 20-25% and 35-40%, respectively. At present, India imports 30% of nitrogenous, 70% of phosphatic and 100% of potassium fertilizer. Both N and P fertilizers are highly energy intensive and at the same time also have very low use efficiency. In addition, there are several drawbacks in the prevailing practices of nutrient management such as non-judicious blanket nutrient application, skewed



NPK ratio, and nutrient mining etc., that pose severe threats to the productivity and sustainability of intensive rice production systems. At the same time inappropriate use of these nutrients has several socioeconomic and ecological consequences such as enhanced fertilizer cost, fossil fuel burning, greenhouse gas emission, pollution of water bodies etc. Therefore an appropriate nutrient management strategy apart from enhancing nutrient use efficiency, productivity and profitability should also aim at enhancing eco-efficiency and environmental sustainability.

Voluminous research has been done to develop and optimize appropriate nutrient management strategy for rice and rice based systems in varying agro-ecological conditions. Most of the early researches focused on broad based blanket nutrient recommendations for similar agro-climatic region. However, these recommendations did not consider field-to-field variability of soil nutrient status which is often led to either excess or deficit nutrient application resulting in loss of nutrient, reduced yield poor nutrient response and low nutrient use efficiency. During past few years tremendous progress has been made in the nutrient management research in order to satisfy "4 R" criteria i.e. right dose, right time, right source and right place, required for enhancing nutrient use efficiency. This led to development of numerous tools and technologies that can be used in rice cultivation across the agro-ecosystem such as soil test crop response (STCR) based N, P, K recommendation, Site Specific Nutrient Management (SSNM) using omission plot technique and targeted yield approach, Real Time N Management (RTNM) using leaf colour chat, chlorophyll meter and green seeker, use of enhanced efficiency fertilizer materials (EEFs) such as urea super granules, coated urea, and nano-fertilizers etc.

Considering the fact that relationship between soil fertility status, nutrient use efficiency and yield at farm level is highly scattered and show great degree of variation, and a common nutrient management strategy may not be appropriate for farmers of different agro-ecology and socioeconomic background. Efforts have been made to upscale the data base with respect to soil fertility management from field to regional level. Accordingly, management zones of rice cultivation have been delineated using GIS, GPS and remote sensing tools.

Nutrient use efficiency depends on plant's ability to uptake nutrient from soil either native or applied and to convert it into final economic product and is controlled by complex interactions of physiological, developmental and environmental processes in soil-plant-atmosphere continuum. Multidisciplinary approach involving agronomy, soil science, microbiology, plant physiology and genetic studies is being followed to identify controlling factors of nutrient use efficiency of rice, developing efficient genotypes and devising appropriate nutrient management strategy (Fig. 1). Apart from that nutrient management research requires a thorough understanding of fate of nutrients in soil, water, plant and atmosphere in emerging scenarios of climate change, development of next generation rice (super rice, high protein rice, multi abiotic stress tolerant rice etc.) and promotion of conservation agricultural practices for bringing in new innovations in the management of N,P,K and micronutrients in rice.







Objective of this chapter is to discuss about the progress, which have been made so far in management of nutrients for enhanced productivity and nutrient use efficiency of rice and rice based production system, and how to fine tune the existing technology for its

Fig. 1.Graphical presentation showing multidisciplinary approach for understanding regulatory factors of nutrient use efficiency.

wider adaptability. This chapter will also discuss about the need of redefining nutrient management strategy in the context of climate change, next generation super, high protein and abiotic stress tolerant rice.

# 2. STATUS OF RESEARCH

#### 2.1. Nitrogen

Nitrogen is one of the most essential and most limiting nutrient for rice production and application of synthetic N fertilizer plays a crucial role in enhancing the yield. Globally rice cultivation consumes approximately 9 to 10 million tons of fertilizer N in a year which accounts for about 10% of the total fertilizer N production in the world. However, only 30 to 40% of the applied N is recovered by the crop resulting in large losses of reactive N, which not only negatively affects yield but also drains national exchequer and pollutes environment simultaneously. Cost of remediation of the socioenvironmental side effects of N pollution such as global warming, ground water pollution and eutrophication etc. is huge. Hence, enhancing N use efficiency of rice

has always been a researchable topic for both plant nutritionist and environmental scientists.

Studies on N management in rice mostly revolve around '4R' principles of nutrient application i.e. right fertilizer source, right dose, right time and right place (Fig. 2).

Among inorganic sources of N, urea is the most widely used nitrogenous fertilizer in rice because of its high N content and favorable physical properties. However, the major

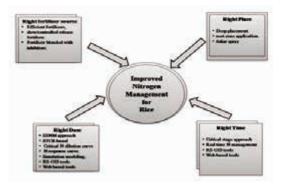


Fig.2. Four "R" approach of nitrogen management for enhanced N use efficiency in rice.



disadvantage with urea is that once applied to waterlogged soils of low land rice, it undergoes rapid transformation processes such as hydrolysis, nitrification, denitrification, leaching and volatilization etc. resulting in loss of up to 50% of applied urea N.

Efforts have been made to develop slow release or controlled release urea fertilizers by coating urea prills with less soluble chemicals such as sulfur, polymers and other products like plaster of paris, resins and waxes. These coated urea products were tested both in laboratories and in field condition with varying degree of effects on urea hydrolysis and N recovery efficiency. Besides this several chemical and natural inhibitors for inhibiting and/or slowing down the hydrolysis of urea (urease inhibitors: NBPT - N-(n-butyl) thiophosphoric triamide - Agrotain, N-phenyl phosphoric triamides (2-NPT), Hydroquinone (HQ), Phenyl phosphorodiamidate (PPD/PPDA)) and biological oxidation of ammonical-N to nitrate-N (nitrification inhibitors: Nitrapyrin, DCD, N (2,5 dichlorphenyl) succinic acid monoamide (DCS), 3,4-dimethylpyrazole phosphate (DMPP)) have been identified and evaluated. The fertilizer products with the coatings of less permeable material and one or more inhibitors as extra additive within the formulation or as in the coating are known as enhanced efficiency fertilizers (EEFs). The EEFs are generally designed to regulate either nitrification or urea hydrolysis or both in order to reduce N loss and increase N uptake by plant. A comprehensive analysis of hundreds of studies all over world with respect to effectiveness of different EEFs showed that urease inhibitors could increase yield and N use efficiency up to 9% and 29%, respectively and reduce in N loss up to 41% in rice-paddy system (Li et al. 2017).

Presence of thin oxidized layer overlying reduced zone in soil is one of the reasons behind rapid loss of N from the rice ecosystem in various forms when it is broadcasted to surface soil. Studies on right place of N application indicated appropriate method or place of N application may vary according to time and source of N application. Basal incorporation of urea in puddled soil of transplanted rice has been observed to reduce NH, volatilization as compared to surface broadcasting of urea to flooded soil, but its impact on yield depends on other factors like water management and tillage practices during incorporation. Inter-row band application of urea during top dressing in direct seeded rice and deep (5-7 cm below surface) placement of USGs in reduced zone in transplanted rice was found to be superior in terms of enhancing N use efficiency and reducing N loss over surface broadcasting of urea. Considering the drudgery and labor involved with manual deep placement method, several attempts have been made to develop continuous operation type and non-continuous injector type USG applicator for both basal and top dressing in transplanted rice. These applicators, however, need to be fine-tuned to make those more user friendly and efficient with respect to metering and uniform depth of application. Technique of injecting dissolved urea into the upper soil layer, has also been developed which is equally effective as deep placement of USG and at the same time less laborious and can be used for top dressing too. Recent study on one-time root zone fertilization (RZF) technique showed that basal application of urea into 10 cm deep holes dug at a distance of 5 cm from the rice roots reduced fertilizer-N loss by 56.3-81.9% compared



to urea surface broad casting (Liu 2016). In addition to soil application, foliar spray of urea has been suggested to avoid the complex interactions of urea in flooded soil of rice. Its effect on grain N content mainly cultivar specific and varies with time of application, however spraying of urea at flowering stage led to a more efficient dry matter partition to the grain, higher grain number m<sup>-2</sup> and finally increased grain yield (Sarandon and Asborno 1996).

Synchronization of N supply with that of crop N demand is the key for enhancing N use efficiency of crop, deciding time and rate of N application is an area of active research in the field of nutrient management in rice. Timing of N application or the decision on split application of N depends on the N requirement pattern of the rice plant. The N absorption in early duration rice varieties is continuous from transplanting to flowering after which there is almost no absorption. In medium and late duration varieties the dry matter and N accumulation is vigorous from transplanting to maximum tillering stage after which it slows down during the vegetative lag phase and again becomes vigorous with onset of panicle initiation and continues little after flowering. Thus there is a single peak of N requirement in early varieties and two peaks of N requirement in medium and late duration varieties, suggesting the importance of basal N application in early varieties and split application in medium and late duration varieties. The number of split is also decided on the basis of soil texture. In soils of relatively finer textures, N is applied in three splits with 50% at basal dressing, 25% at 21 days after transplanting (DAT) and the rest 25% at panicle initiation. But in soil of lighter textures, N application in three splits of 25:50:25 proportions are considered best. For hybrid rice, N is applied in four equal splits doses- 25% of N at basal dressing, 25% at 21 DAT, 25% at PI and the rest 25% at panicle emergence. However, in rainfed lowland direct seeded rice the entire dose of 60 kg N ha<sup>-1</sup> along with 30 kg  $P_2O_5$  and 30 kg K<sub>2</sub>O ha<sup>-1</sup> is applied in seed furrows at the time of dry sowing. Response of crop to applied N is highly field specific and varies with soil condition hence correct N recommendations requires information on N availability from all possible sources and crop requirement. The site specific N management (SSNM) recommendation based on indigenous N supply, expected N demand of crop and the expected fertilizer N use efficiency resulted in an increase in N-use efficiency of irrigated rice by 30-40% and grain yield by 7% in more than 100 field experiments in Asia (Dobermann et al. 2002). Going a step further, ways and means were also devised to address the real time need of crop which generally varies according to growth stage and environmental condition. Hand held optical sensors such as chlorophyll meter, green seeker etc. are promising real time N management tools which indicate crop N status non-destructively on the basis of greenness of leaf. Recently leaf colour chart (LCC) is being widely tested and promoted as an easy to use cheap and farmer's friendly diagnostic tool of real time N application. The extensive field research in several parts of Asia indicated up to 25% saving of N in rice production could be achieved by using LCC.

Crop simulation based decision support tools such as DSSAT, ORYZA-2000, Info Crop have been developed to help determine N fertilizer recommendations matching



with crop requirement. These tools consider complex interaction of N transformation processes in soil-plant-atmosphere continuum and are useful to predict N management option in different scenarios of agronomic management. Development of light and portable hyper-spectral sensors and new generation satellites which obtain higher resolution images offer the possibility to detect in season N status of crop on the basis of the normalized difference vegetation index to provide recommendation for larger areas at affordable prices.

Nitrogen fertilizer is an integral component of green revolution in India and most of the initial researches on N recommendation were related to agronomy trials on yield response in different agro-climatic zone. Few studies on site specific N recommendation have been initiated in several rice growing regions like Punjab, Odisha, Bihar etc. Rice Crop Manger is such an initiative by IRRI in collaboration with ICAR institutes that gives field specific N recommendation on the basis of past crop management history. Recently Bijay-Singh et al. (2015) recommended a moderate amount of N application at transplanting, followed by sufficient N fertilization at active tillering, and an optical sensor-based N application at panicle initiation stage for enhanced yield and N use efficiency in transplanted rice. However, the real time N management tools like chlorophyll meter and green seekers etc. are confined to experimental purposes only. Extensive studies on standardization and evaluation of leaf colour chart based in season N application have been conducted in farmers' fields in different parts of India.

Studies on use of EEFs for enhancing N use efficiency of rice in India are limited to experimental stations only. Several attempts have been made for identification and field scale evaluation of plant based natural inhibitors. Nimin, a tetraterpenoids extracted from neem (*Azadirachtaindica*) cake and karanjin, a flavonoid obtained from the seeds of the karanj (*Pongamiapinnata*) are reported to have nitrification inhibition property. Technology has been developed to produce Neem coated urea by coating urea prills with neem (*Azadirachtaindica*) oil emulsion. Field trials with rice indicated reduced N loss, enhanced yield and N use efficiency with NCU as compared to normal urea. Today according to the order of Government of India all urea manufactured in India are coated with neem oil emulsion.

Development of appropriate N management strategy has always been an integral component of rice research at ICAR-NRRI. Number of studies has been conducted to understand fate of urea in low land rice under different improved N management options. The time of complete urea hydrolysis in irrigated and rainfed rice ecosystems was found to be 2-3, 5-7, and 7-14 days for broadcasting of urea onto flooded soil, deep placement of USG in flooded soil and urea basal application in dry soil, respectively (Nayak and Panda 2002). Relative ammonia volatilization and surface runoff loss from rice field was estimated to be 0.4% and 6-8%, respectively with USG deep placement and 6.0% and 78% with urea broadcasting on waterlogged soil (Nayak and Panda 2002). Subsurface placement of urea through incorporation of applied urea into wet soil by puddling in absence of any standing water and deep placement urea mud balls in the reduced zone of submerged rice soil, also decreased N losses and improved N



use efficiency in rice (Kabat 2001). Coating of urea with neemcake or shellac for basal dressing also reduced ammonia loss (Mishra et al.1990). Nayak and Panda (1999) tested efficacy of different nitrification inhibitors found that hydroquinone was more effective in alluvial and laterite soils and alcoholic extract of neem cake was better in black soil than dicyandiamide. In direct seeded rice, seed furrow placement of urea produced higher grain yield than broadcasting method.

Yield response trials were conducted to optimize N dose for semi-dwarf rice in wet season (40-80 kg ha<sup>-1</sup>), rainfed lowland direct sown rice varieties, (56-62 kg ha<sup>-1</sup>) and hybrid rice (100 kg N ha<sup>-1</sup> in *kharif* and 120-135 kg N ha<sup>-1</sup> in *rabi* season). In addition to this, nitrogen accumulation pattern of different rice cultivars was investigated in different agro-ecological conditions to ascertain the pattern of N requirement and decide number and time of split N application accordingly. Efforts have been made to optimize N doses for several medium to long duration rice varieties grown at different planting dates by crop simulation approach using of ORYZA 1N model.

Recently a five panel customized leaf colour chart (CLCC) was developed for real time nitrogen management in rice of different agro ecologies by ICAR-NRRI. The CLCC provides cultivar specific recommendation of basal as well as top dressing of N (both time and dose) in terms of kg urea per acre for rainfed favorable lowland, submerged and flood prone lowland, rainfed upland, and irrigated rice (Nayak et al. 2013). Field trials indicated yield advantages of 0.5-0.7 t ha-1 with CLCC based N application over recommended practice. Application of neem coated urea (NCU) on the basis of CLCC reading enhanced yield and N recovery efficiency (REN) by 21.2-22.9% and 16.3-18.0%, respectively, over conventionally applied urea (RDF-Urea) in aerobic direct seeded rice (DSR) and by 14.6-15.9% and 11.6-14.6%, respectively in puddled transplanted rice (PTR). Further, in aerobic direct seeded rice, NCU when applied on the basis of leaf colour chart reduced NO<sub>2</sub>-N leaching and N<sub>2</sub>O-N emission by 26% and 11-21%, respectively as compared to conventionally applied urea (Mohanty et al. 2017). Web based nutrient management tool-rice crop manager (RCM) was developed in collaboration with IRRI to give field specific recommendation for the farmers of Odisha on the basis of past cropping and management history information. Rice crop manager recommendation provided rice grain yield advantage of 9.8 to 39.6% with an average of 22.6% over farmer's practice (FFP). Remote sensing and GIS tools were used to provide recommendation of N application for homogenous management zone in few selected pockets of Odisha. Site specific N recommendations of 66-100 kg ha<sup>-1</sup> with an average of 80 kg ha<sup>-1</sup> were computed using historical soil data, yield target and expected agronomic N use efficiency. Site specific fertilizer N recommendation (SSFN) map was generated for Ersama block, Jagatsingpur district of Odisha using appropriate semi-variograms and interpolating SSNM values by kriging.

Research has been initiated at ICAR-NRRI to fine tune technique of urea briquette deep placement to make it more efficient and user friendly. The breakability of the briquettes was reduced by mixing urea with oils of neem (*Azadirachta indica*) and karanj (*Pongamia pinnata*). Apart from being good binding agent, the oils used



contain active ingredients that reportedly inhibit nitrification activity in soil. Mixing oil increased the strength of briquettes and reduced the breaking percentage to 2-5% as compared to 25-30% of urea briquette without a binding agent. In addition to this, agglomerated urea briquettes were prepared by mixing suitable amendments viz. phospho-gypsum, fly ash, silica powder, neem cake and rice husk as filling materials and biodegradable binding agents with urea (Nayak et al. 2017). Use of amendments acted as filler material and reduced the concentration of urea in pellet which will ensure its uniform distribution in the field. Efforts were also made to improve the existing urea applicator and develop new prototypes. Manually pulled 2, 3 and 4 row drum type urea briquette applicators for basal application, briquette applicator mounted on conoweeder for top dressing, injection type briquette applicator for both basal and top dressing are fabricated and tested in the field.

## 2.2. Phosphorous

About 50% of agricultural soils, on a global scale, are suffering from deficiency of phosphorus (P), which has been majorly observed due to two prime factors, (i) insufficient P replacement in agricultural soil, and (ii) P-fixing properties of a particular soil causing P unavailability to plants. In highly weathered soils around the world, mostly belongs to the orders Oxisols and Ultisols, P deficiency has been noticed as a common factor hampering the agricultural production. Apart from the inherent soil conditions to supply P, large imbalances in the rates of P fertilizer also exist, showing the variations of lower or inadequate P application in many developing countries of Asia, Africa and South America but adequate or excess P application in the Europe, USA and few Asian countries (China, Japan and Korea) as reported by few analysis over the years.

Response to P application is highly erratic due to direct and indirect influences of several factors operating in the soil system on P availability to the plants. In general, the response of lowland rice is usually lower than other dryland crops including upland rice. Reduced soil conditions normally increase the P availability to lowland rice. Therefore, in many soils, P availability is not a yield-limiting factor for rice and significant response of modern rice varieties to fertilizer P may be observed after several years of intensive cropping. Thus management must focus on the buildup and maintenance of adequate available P levels in the soil, so that P supply does not limit crop growth. P fertilizer applications exhibit residual effects thus maintenance of soil P supply requires long-term strategies on site-specific basis. Organically amended plots (FYM, rice straw, and green manuring) in eastern India showed an improved PUE than control. While others have found that effect of FYM application on P use efficiency was not very prominent in irrigated rice rather it helps in recycling P. Application of phosphorous solubilizing bacteria (PSB) to lowland rice ecology had no significant impact while addition of phosphatic fertilizers and vermicompost governed the soil P availability in the same occurrence (Kumar et al. 2016). Upland rice-based crop rotation with maize and horse gram promoted native arbuscular mycorrhizal fungi (AMF) colonization (in a tune of 10.4-38.8%) and ensured better P



uptake (2.2–2.6 mg P g<sup>-1</sup> plant) by rice crop which further reflected in higher grain yield (Maiti et al. 2012).

Better phosphorus use efficiency (PUE) can be addressed through two clear-cut approaches: 1. Genetically P efficient cultivars, and 2. P management by external means. Scientists working in rice crop globally follow similar ways to enhance PUE. Genotypes grown well at low soil P level but responded well to added P is the most desirable trait for screening P efficient rice genotypes. The study further identified two factors, shoot weight and P uptake, are the most crucial to identify P deficiency. Moreover, PUE was identified higher in roots over shoots in rice grown under acid soils that decreased with increasing levels of soil P (Fageria and Baligar1997). By cutting edge approach of recent times, scientists have explored the possibility of genetic manipulation in rice for improved PUE, including the identification of *PSTOL1* (phosphorus starvation tolerance 1) gene, which is a key gene responsible for the natural variation in phosphorus starvation tolerance, induce enhanced grain yield in P-decient soil by promoting early root growth and Pi acquisition, over a range of intolerant and tolerant rice genotypes like Kasalath (Gamuyao et al. 2012).

#### 2.3. Potassium

In tall *indica* rice, there was limited response to K application in earlier years, but with the introduction of high yielding varieties and intensive agriculture, response in grain yield was recorded. The mean response of rice over several years in intensive cropping systems of the long term fertilizer experiments ranged from 4 to 10 kg grain for every kg of K<sub>2</sub>O applied. In view of high uptake of K by the high yielding varieties of rice, application of a maintenance dose of 30 kg K<sub>2</sub>O ha<sup>-1</sup> for lowland rice is suggested. In light textured acid upland soils, K deficient areas, in biotic and abiotic stress situations and in hybrid rice, split application of K or K top dressing along with N at panicle initiation in addition to a basal dressing of K is beneficial for increasing rice yield. Rice is mostly cultivated under submerged condition. Under such condition potassium fertility level changes immediately after inundating the soils due to release of soluble ferrous (Fe<sup>2+</sup>) and manganous (Mn<sup>2+</sup>) ions. These ions displaced K in the exchangeable pools and released into the soil solution. Thus, availability of K increased after submergence. However, contrasting reports are also available showing decrease in K content due to the formation of sparingly soluble Fe-K complexes. Hence, application of potassium along with other nutrients is very important for sustaining rice yield.

Application of inadequate and unbalanced fertilizer excluding potassium is one of the reasons for declining K nutrition in rice fields. In a long-term study in China, it is observed that proper potassium fertilizer management control rice yield in the double rice cropping system (Liao et al. 2013). It was further observed that the yield of both early and late rice increased over time in the treatments that received fertilizer K or combined application of fertilizer with rice straw. Decline in yield of rice is reported when unbalanced fertilizers are applied (Liao et al. 2013). Rice removes large amount of K from soil. Due to high K removal, a negative K balance prevail in rice and rice



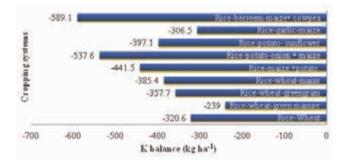


Fig. 3.Potassium balance in rice-based cropping systems (modified after Sharma et al. 2008).

based cropping system even after recommended fertilizer application. Sharma et al. (2008) reported a net negative K balance for nine different ricebased cropping systems (Fig. 3).

Rice straw is a rich source of K, its incorporation into

soil at the rate 5-10 t ha<sup>-1</sup> markedly increases available K content and improves K nutrition of rice crop. Therefore, effective straw management by returning a considerable portion back into the field is another option for effective K management (Bijay-Singh and Singh 2017). Split application of potassium in rice field increased the yield and potassium use efficiency of rice in light textured soil. However in a long term fertilizer experiment at NRRI, response to applied K was observed after 30 years of rice-rice cropping, nevertheless treatment effects were most prominent on release threshold concentration (RTC), followed by cumulative K release, K-release rate constants, and K-fixation capacity. Rice cultivation without K fertilizer application resulted in lower values of soil K parameters than the K fertilized treatments (Debrup et al 2018).

## 2.4. Micronutrients

Micronutrients more particularly Zn, B and Fe play key roles in growth and metabolism of rice plant hence are essential for enhancing yield of low land rice. Zinc deficiency is the most commonly observed micronutrient disorder in rice based cropping system. In contrast, Fe toxicity is the major problem in the most wetland rice soil in humid tropical regions of Asia due to drop of redox potential under submerged condition which elevates the release of Fe(II) in the soil. However, in upland and aerobic rice systems Fe could be a limiting nutrient for rice yield. Additionally, nutrient mining and emerging trend of micronutrients deficiency in soils of many intensively cultivated rice growing regions warrants for a judicious and need based micronutrient application strategy in rice cultivation. Study on effect of micronutrient application on rice yield showed number of tillers per square meter, spikelets per panicle and paddy yield was maximum with combined use of zinc and boron and 1000-grain weight was recorded highest where all three micronutrients (zinc, boron and iron) were applied in combination. The maximum healthy kernel percentage was recorded where zinc was applied along with iron (Qadir et al. 2009).

Flooding the soil alters availability of micronutrients like Fe, Mn and Zn which interact among themselves and determine their uptake by plant. However, the soil test





based approach is considered best for application of these nutrients. Among the different available Zn fertilizers  $ZnSO_4$  is the most commonly used Zn fertilizer because of its high solubility. In Zn deficient soil one time application of 20 to 25 kg  $ZnSO_4$  ha<sup>-1</sup> as basal has been recommended. However foliar spray of 0.5%  $ZnSO_4$  in 200 litre water ha<sup>-1</sup> has been suggested in emergency condition of Zn deficiency during rice growth period. In calcareous soil incorporation of Zn fertilizer with green manure is generally considered better than broadcasting.

Apart from upland condition, Fe deficiency also occurs in, calcareous, and alkaline low land soil with low organic matter content. Management practices like application of organic manure before sowing and green manuring with dhaincha before rice transplanting have been proved beneficial to alleviate Fe deficiency in rice. Ponding of water in nursery beds during dry spell and irrigating rice fields as soon as chlorosis appears have also been suggested to deal with Fe deficiency. In addition to this broadcasting of FeSO<sub>4</sub>.7H<sub>2</sub>O at the rate of 30 kg Fe ha<sup>-1</sup> as basal and spraying 1% FeSO<sub>4</sub>.7H<sub>2</sub>O solution 2-3 times at 10 days interval have been recommended. Application of lime (75% LR) along with Zn and other limiting elements such as K and Mn ameliorated iron toxicity in some lowland iron rich rice soils (Shahid et al. 2014).

Manganese deficiency mainly occurs in alkaline and sodic soil with low organic matter, leached and acid sulphate soils and degraded soils containing large amount of soluble iron. Integrated application organic manures like FYM, compost or green manure along with 25 kg  $MnSO_4$  ha<sup>-1</sup> as basal could be the best management strategy to address the problem of Mn deficiency in rice. In addition, spraying of 0.5%  $MnSO_4$  has been recommended for rapid correction of Mn deficiency.

Rice grown in highly weathered, acid upland, coarse textured sandy soils and calcareous soils mostly suffers from B deficiency. Generally, soil test based application of borax at the rate of 5-10 kg ha<sup>-1</sup> as basal has been recommended for B deficient soil. However, spraying of 0.2% boric acid or borax at pre flowering or heading stages has been proved to be effective in taking care of hidden deficiency. For quick recovery from B deficiency, spraying of borax or boric acid at the rate of 0.05% has also been suggested.

#### 2.5. Integrated nutrient management

Integrated approach of using both organic manures (Farm yard manure (FYM), green manure (GM), crop residues, biological  $N_2$  fixation and biofertilizers) and chemical fertilizers have been often recommended for managing soil fertility and nutrients in intensive rice based cropping system to arrest the trend of yield stagnation and minimize adverse environmental impact of chemical fertilizers. Numerous studies have been conducted to ascertain the beneficial effects of organic manure application on soil health and subsequent positive impact on growth and yield of rice. Sustainability yield indices calculated for intensive rice production system often show comparatively higher value when part of the recommended nutrient is applied through organic sources such as FYM or GM than chemical fertilizer alone. Continuous application of chemical fertilizers along with FYM to a 44 year old rice-rice system in tropical India



resulted in improvement in soil physical and chemical properties and biological activity leading to higher soil quality index and greater sustainability (Shahid et al. 2013). Studies conducted at CRRI, Cuttack revealed that growing Azolla before rice transplanting or after transplanting produces an additional grain yield of more than 0.5 t ha<sup>-1</sup> and is equivalent to application of 30 kg of fertilizer-N. Free living bacteria Azotobacter can fix 10-30 kg N ha<sup>-1</sup> in aerobic soil, the associative bacteria Azospirillum can fix 7 kg N ha<sup>-1</sup>; with its inoculation, grain yield increased by 8-30% over the uninoculated one.

# 3. KNOWLEDGE GAPS

Despite volumes of research and adoption of innovative technology, the N use efficiency of low land rice has not been improved substantially. The recent approach of SSNM along with RTNM has the potential to ensure efficient utilization of applied N; however these technologies need to be simplified while retaining their effectiveness to ensure large scale adoption by the small and marginal farmers growing rainfed rice. Deep placement of urea super granules/urea briquettes in the reduced zone has been proved to enhance N use efficiency and decrease N loss but in absence of a cost effective efficient applicator for uniform application, deep placement technology has not made any desired impact. Quantitative understanding of the complex interacting processes taking place in soil as well as in plant that influence N use efficiency of rice is still insufficient. Source sink relationship of super rice/next generation rice and high protein rice is inadequately understood. Methods for estimation of various N loss from rice ecosystem are yet to be standardized and up-scaled in different agroclimatic conditions, the limited data generated following these methods are associated with great degree of uncertainties. The fate of USG deep placement in light sandy loam textured soil and in direct seeding rice in unpuddled soil, where substantial amount of leaching takes place has not been properly known. Integrating satellite based real time monitoring of soil and plant nutrient status with weather forecasting for fertilizer recommendation advisory is another potential area of research. Similarly, microbial processes like anaerobic ammonia oxidation and phyllospheric N fixation which could have a bearing with N loss and N use efficiency of urea deep placement is poorly understood.

Phosphorus is a unique element and indispensable for sustenance of crop plants, microbes and ecosystem. In soil system major form of P is phosphate  $(PO_4^{3-})$  which binds with the available cations  $(Ca^{2+}, Mg^{2+}, Al^{3+})$ ; however where rice grows in a typical conditions (lowland, puddled, submerged, anaerobic) the availability of P and its dynamics over time with changing pH and redox potential (Eh) is a researchable issue. Moreover, the interaction of P ions with other abundant major (N, K) and micronutrient ions (Fe, Zn, Cu, Mn) demands attention under low land/submerged situation.

The major concern in K research is whether the ammonium acetate extractable K is sufficient to justify the K supply for plant need. At present, additional parameters like





the two categories of non-exchangeable K reserve *viz.* step-K and constant rate K and the release and fixation threshold levels of K are important issues. Till date, there is not much report available on the long-term effect of differential nutrient management on potassium supply parameters under different cropping system. Role of micro and macronutrients including K for ameliorating the biotic and abiotic stress in the context of climate change is being focus of current nutrient management research.

Studies indicated microbial inoculants can be efficiently integrated in a nutrient management programme to reduce chemical fertilizer input and enhance nutrient use efficiency; however the effectiveness and stability of the inoculants under diversified agroclimatic condition is yet to be properly ascertained.

## 4. RESEARCH AND DEVELOPMENT NEEDS

#### 4.1. Up scaling N recommendation

Though the leaf colour chart is being widely popular among the farmers as a easy to use diagnostic tools for in season N application, it still is a qualitative indicator of crop N status and provides a non-quantitative recommendation of N application and not helpful for regional scale recommendations. More recently, some non-invasive optical methods based on absorbance and/or reflectance of light by the intact leaf, have been developed. These include ground-based remote sensors and digital, aerial, drone and satellite imageries, which can be used for regional scale and quantitative recommendations to overcome tedious soil and tissue testing. The remote sensing (RS) based techniques have a great potential in formulating cost effective N recommendations for reducing fertilizer doses and environmental risks and improving nitrogen use efficiency and crop yield. But a very limited work has been done in this field, as they have a greater future scope, emphasis should be given towards improving and promoting these techniques. Remotely sensed data collected from plant canopies can be used to formulate vegetation indices that may give an indication of plant health. Several vegetation indices have been developed including, Normalized Difference Vegetation Index (NDVI), Green Normalized Vegetation Index and infrared to red reflectance ratio or simple ratio (SR). This provides opportunity for defining fertilizer N algorithm for optimum N recommendation in different rice growing regions of India on the basis of expected yields and achievable greenness of the leaves using NDVI during the crop-growth period. Research can be directed to delineate N management zone for rice indicating deficit, optimal and surplus region on the basis of RS based Nitrogen Nutrition Index (NNI) approach by taking into consideration the measured N concentration and predicted critical N concentration during growing season.

#### 4.2. Enhancing phosphorus use efficiency

Enhancing phosphorus use efficiency (PUE) and exploring the possibility of harnessing the maximum share of P for crop nutrition from conventional (common phosphatic fertilizers) and non-conventional fertilizer (oilseed cake, bone meal, fish



meal, rock phosphate etc.) sources is essential to reduce the dependence on phosphatic fertilizer for rice cultivation. Research is needed to exploit the potential of beneficial soil microbes like AMF alone or in combination with PSMs for improving crop productivity and reduced use of P-fertilizers. Interdisciplinary approach should be followed to investigate the factors affecting P nutrition of rice crop for better understanding of physiological/molecular basis, screening of better P-efficient cultivars and simultaneously devising their management strategies to overcome the problem of P deficiency. More research is needed to develop site specific nutrient management practices for P nutrition in rice that can be easily adopted by the farmers.

# 4.3. Harnessing microbial resources for enhancing use efficiency of potassium and micronutrients

Microbes play a significant role in cycling of nutrient in soil-plant-atmosphere continuum. Strains of beneficial microbes that directly influence availability of nutrient such as potassium solubilizing bacteria (KSB), Zn-solubilization microbes, siderophores producing microbe have been identified. Most of the studies on these microbes are confined to laboratories; research is needed to explore the possibility of using these bacteria to enhance the use efficiency of both applied and inherent nutrients in field scale. In addition, application of improved molecular tools (metagenomics, q-PCR etc.) is needed to explore the untapped potential of rhizospheric and phyllospheric microbial resources for their utilization to enhance nutrient use efficiency of rice.

#### 4.4. Nutrient management under abiotic stress

Research has been carried out to develop single and multi abiotic stress tolerant rice cultivars that can withstand drought, submergence or both to some extent. Advent of submergence tolerant varieties like Swarna Sub1, IR-64 Sub 1 etc. make it necessary to devise appropriate nutrient management strategy to enhance productivity and reduce the yield loss. Some attempt has been made to develop nutrient management recommendation for submerged rice of flood prone areas of eastern India and found that application of additional (20%) basal P and post submergence N application either as soil application or foliar spray (48 h after de-submergence) along with additional potassium enhanced the submergence tolerance of both Sub-1 introgressed HYV and its recurrent parent. More research is needed to understand the dynamics of different nutrients in soil-plant system under various stress conditions and explore the possibility of revival of stress affected crop through nutrient supplementation. Till now most of the nutrient recommendations generated address only single stress condition such as flood, drought and salinity. However, with emerging trend of frequent occurrence of climatic extreme events it is essential to direct our research towards developing appropriate nutrient management practice under multi abiotic stress condition

#### 4.5. Nutrient management research in a changing climate scenario

Climate change variables including precipitation (amount and distribution), temperature and atmospheric  $CO_2$  concentrations change rice productivity. Agricultural productivity is potentially changed by associated changes in crop nutrient use.





Understanding of crop-specific needs for achieving expected yields and soil-specific nutrient supply characteristics is the primary basis for nutrient management recommendations. In present scenario it is important to study the expected changes in ambient CO, concentration, temperature and precipitation which are expected to influence the agriculture. Increases in air temperature and changes in precipitation will significantly impact prevailing root zone temperature and moisture regimes. Nutrient availability, root growth and development are primarily affected by soil moisture and temperature. Limited work have been done to understand N and P dynamics in soil and their subsequent acquisition by crop under elevated CO<sub>2</sub> and temperature condition, however, the nature and extent of the change in these two parameters is highly site- and soil specific. At the same time little information is available regarding impacts of elevated CO, on the nutrient concentrations in solutionphase, whose availability will also be indirectly mediated by temperature and moisture changes. Research is needed to investigate the impact of elevated CO<sub>2</sub> and temperature on dynamics of different nutrient element in soil, availability and mechanism of their acquisition by plant.

#### 4.6. Nutrient management for next generation rice

Development of next generation rice based on the ideotype concept with a yield target of 10-12 t ha<sup>-1</sup>(super rice) and high protein rice with protein content 10-12% are frontier area of research in rice improvement. New plant ideotype necessitates appropriate nutrient management practices for realizing the potential yields. Nutrient response studies are needed for both super and high protein rice to ascertain their requirement of different macro and micronutrients. Crop simulation models such as WOFOST, QuEFTS (Quantitative Evaluation of the Fertility of Tropical Soil) and Nutrient Decision Support System (NuDSS) will be helpful to calculate the nutrient need of super rice on the basis of potential yield. Understanding source sink relationship and site-specific nutrient management are promising options to estimate the nutrient requirements of super rice based on attainable yields and indigenous nutrient supply.

Studies indicated that amount of N uptake in super rice varieties could be as high as 18-20 kg N t<sup>-1</sup>of grain yield and most of the super rice is bred for a high N input condition. Similarly, in case of high protein rice the N requirement could be different than the normal rice. Uncertainties in the crop requirements for N, P and K and other micro-nutrients may result in either excess or deficit application of fertilizers leading to soil nutrient imbalances and associated negative environmental impact. Research is needed to monitor the long term impact of cultivation of these next generation rice on soil health and sustainability and at the same time efforts should be made to develop appropriate nutrient management strategy that ensures environmental sustainability while achieving its potential yield.

## 5. WAY FORWARD

Most of the straight and complex fertilizers currently being used in rice cultivation are in use for last 50-60 years. Research is needed to identify and develop cheap chemical and organic source of plant nutrients particularly customized fertilizer



products specific to crop and region. Blanket recommendations of fertilizers for rice at state and national level are in practice since long time. At present the nature of soil, type of varieties, and environmental conditions have been changed from the time when the fertilizer recommendation was made. Therefore, revised recommendation of fertilizers are required keeping in mind the high level of exhaustion of soil nutrients by high yielding varieties.

Progress has been made so far from blanket crop response based approach of nutrient recommendation to need based site specific nutrient recommendation. However, to enhance eco-efficiency of applied nutrient and minimize negative environmental impact, it is essential to adopt ecological intensification based nutrient management approach which takes into consideration ecological processes and the beneficial interactions among different components of agro-ecosystem.

The 4 'R' stewardship approach of nutrient management need to be relooked in the context of development of sensor based precision real time monitoring system and advent of next generation super, high protein, biofortified and climate resilient rice. In the context of climate change, nutrient management strategy for enhancing tolerance to biotic (disease and pests) and abiotic (drought, submergence, high and low temperature) stresses need to be devised. In addition to this, it is essential to develop nutrient management strategies for low input rice farming particularly in difficult ecology.

Some emerging technologies like nano-technology, seed coating, liquid organic fertilization etc. have potential to bring about substantial improvement in nutrient use efficiency of arable crops. Both strategic and basic research is required to explore the possibility of using nano-fertilizers for N and P nutrition of rice and at the same time assess its undesirable side effects on soil flora and fauna. Liquid organic byproducts of bioreactors that produce bioethanol has been identified as a good source of nutrient and its use as a fertilizer is increasing in many developed countries. Recycling bioreactor waste as a source of nutrient in rice production requires systematic research and development support and involvement of extension machinery.

Production of EEFs in India is very limited, few companies produce SCU, PCU in a small scale and inhibitors are mostly imported. Non availability of these products and associated high cost prevent their wide scale use in rice cultivation. Government policy support in form of fertilizer subsidy could address this problem. Coating seeds with nutrients formulation is a promising technique to enhance nutrient use efficiency and showed positive effect on P and N nutrition however this technique is in nascent stage and require further investigation for its practical use.

#### References

- Bijay-Singh and Singh VK (2017) Fertilizer management in rice. In: B.S. Chauhan et al. (eds.), Rice Production Worldwide, Springer International Publishing. pp. 217-253 doi: 10.1007/978-3-319-47516-5-10.
- Bijay-Singh, Varinderpal-Singh, Purba J, Sharma RK, Jat ML, Yadvinder-Singh, Thind HS, Gupta RK, Choudhary OP, Chandna P, Khurana HS, Kumar A, Jagmohan-Singh Uppal HS, Uppal



RK, Vashistha M. and Gupta RK (2015) Site-specific nitrogen management in irrigated transplanted rice (*Oryza sativa*) using an optical sensor. Precision Agriculture16 (4):455–75.

- Das, D, Nayak AK, Thilagam, V, Chatterjee D, Shahid M, Tripathi R, Mohanty S, Kumar A, Lal B, Gautam P, Panda BB and Biswas SS (2018) Measuring potassium fractions is not ufficient to assess the long-term impact of fertilization and manuring on soil's potassium supplying capacity. Journal of Soils and Sediments https://doi.org/10.1007/s11368-018-1922-6
- Dobermann A, Witt C and Dawe D (2002) Nutrient management in intensive rice cropping systems of Asia. Better Crops International 16 (1):25-30
- Fageria NK and Baligar VC (1997) Upland rice genotypes evaluation for phosphorus use efficiency.Journal of Plant Nutrition 20(4):499-509.
- Gamuyao R, Chin JH, Pariasca-Tanaka J, Pesaresi P, Catausan S,Dalid,Slamet-Loedin I, Tecson-Mendoza EM, Wissuwa M andHeuerS (2012)The protein kinase Pstol1 from traditional rice conferstolerance of phosphorus deciency. Nature 488:535-539.
- Kabat, B (2001) Studies on placement of urea-based fertilizers and their use in integration with biofertilizers in rainfed lowland rice (*Oryza sativa L*.). Ph.D. Thesis, Utkal University, Bhubaneswar.
- Kumar SS, Deb S, Bhadoria PB, Mukhopadhyay D, Rakshit A and Choudhury A (2016) Impact of *Pseudomonas putida* on available soil phosphorus dynamics and crop productivity under lowland rice ecology. Nature Environment and Pollution Technology15(1):227-232.
- Li T, Zhang W, Yin J, Chadwick D, Norse D, Lu Y, Liu X, Chen X, Zhang F, Powlson D, Dou Z(2017) Enhanced-efficiency fertilizers are not a panacea for resolving the nitrogen problem.Global Change Biology.doi: 10.1111/gcb.13918
- Liao Y, Zheng S, Nie J, Xie J, Lu Y. and Qin X (2013) Long-term effect of fertilizer and rice straw on mineral composition and potassium adsorption in a reddish paddy soil. Journal of Integrative Agriculture 12(4):694-710.
- Maiti D, Singh RK and Variar M (2012) Rice-based crop rotation for enhancing native arbuscularmycorrhizal (AM) activity to improve phosphorus nutrition of upland rice (*Oryza sativa* L.). Biology and Fertility of Soils 48(1):67-73.
- Mishra, B, Sharma, D and Murthy, JR (1990) Effect of neem cake and shellac coating of urea and green manure on ammonia volatilization and nitrogen use efficiency for rice. Journal of the Indian Society of Soil Science 38:224-228.
- Mohanty S, Swain CK. Tripathi R, Sethi SK, Bhattacharyya P, Kumar A, Raja R, ShahidM, Panda BB,Lal B, Gautam P, Munda S. and Nayak A K. (2017) Nitrate leaching, nitrous oxide emission and N use efficiency of aerobic rice under different N application strategy. ArchivesofAgronomy and Soil Science.https://doi.org/10.1080/03650340.2017.1359414
- Nayak, SC and Panda, D (1999) Kinetics of nitrification of applied urea in alluvial, laterite and black soils with and without nitrification regulators. Journal of the Indian Society of Soil Science 47:544-546.
- Nayak, SC and Panda, D (2002) Transformation of applied urea and its efficient utilization in rainfed low landrice(*Oryza sativa* L). Fertiliser News47(2):47-58.
- Nayak AK, Mohanty S, Chatterjee D, Guru PK, Lal B, Shahid M, Tripathi R, Gautam P, Kumar A, Bhattacharyya P, Panda BBand Kumar U (2017) Placement of urea briquettes in lowland rice: An environment-friendly technology for enhancing yield and nitrogen use efficiency. NRRI Research Bulletin No.12 ICAR-National Rice Research Institute, Cuttack, Odisha 753006, India. pp 1-26.



- Nayak AK, Mohanty S, Raja R, Shahid M, Lal B, Tripathi R, Bhattacharyya P, Panda BB, Gautam P, Thilagam VK, Kumar A, Meher J and Rao KS (2013)Customized leaf colour chart for nitrogen management in rice for different ecologies.CRRI Newsletter, Jan-March, p. 14.
- Qadir J, Awan IU, Mohammad SB, Shah IH, MuhammadAN, Saba N andBakhsh I (2013) Application of micronutrients for yield enhancement inrice.Gomal University Journal of Research 29(2):9-16.
- Sarandon SJ and Asborno MD (1996) Foliar urea spraying in rice (*Oryza sativa L.*), effects of time of application on grain yield and protein content. Cereal Research Communication 24 (4):507- 514.
- Shahid M, Nayak AK, Shukla AK, Tripathi R, Kumar A, Raja R, Panda BB, Meher J, Bhattacharyya P and Dash D (2014) Mitigation of irontoxicity and iron, zinc, and manganese nutrition of wetland ricecultivars (*Oryza sativa L.*) grown in iron-toxic soil. Clean – Soil,Air, and Water 42 (11):1604–1609.
- Shahid M, Nayak AK, Shukla AK, Tripathi R, Kumar A, Mohanty S, Bhattacharyya P, Raja R, Panda BB (2013)Long-term effects of fertilizer and manure applications on soil quality and yields in a sub-humid tropical rice-rice system. SoilUse and Management29(3):322– 332.
- Sharma RP, Pathak SK, Haque M and Lal M (2008) Productivity, profitability and nutrient balance as influenced by diversification of rice (*Oryza sativa*)-wheat (*Triticumaestivum*) cropping system. Indian Journal of Agronomy (53):97-101.\*



# Assessing Energy and Water Footprints for Increasing Water Productivity in Rice-based Systems

## R Tripathi, M Debnath, S Chatterjee, D Chatterjee, A Kumar, D Bhaduri, A Poonam, PK Nayak, M Shahid, BS Satapathy, BB Panda and AK Nayak

# SUMMARY

242

Water is one of the important elements responsible for life on earth. Globally, three major sectors i.e. agriculture, domestic consumption and industry compete for water. India presently has the world's second largest population and there is a net export of agricultural products from India, which is likely to continue. These developments will lead to a larger water demand for the agricultural sector in near future. Water management is becoming a key issue affecting the availability and distribution of already scarce fresh water to growing population. The data regarding water usage and availability of water is not available which poses a challenge for sustainable management and development of water resources. Hence, measurement and quantification of the energy footprints, water footprints and water balance components are fundamental for understanding the hydrological behaviour of a system for effective water management. The objective of this chapter is to discuss the water footprints of rice in different cultivation practices in comparison to other crops and discussing the key challenges and issues related to the water management and water balance studies particularly in river basins of India and need for advance methodologies for studying water footprint, and energy balance components. Globally, water footprint of rice paddy production is 784 km<sup>3</sup> yr<sup>-1</sup> with an average of 1325 m<sup>3</sup> t<sup>-1</sup>. The mean water footprint of cereals is about 1644 m<sup>3</sup> t<sup>-1</sup>. Among them, the water footprint for millet is comparatively large (4478 m<sup>3</sup> t<sup>-1</sup>), while for maize it is comparatively small (1222 m<sup>3</sup> t<sup>-1</sup>). Water productivity differs with different cultivation systems and irrigation techniques, which is discussed. The mean water footprint of rice  $(1673 \text{ m}^3 \text{ t}^{-1})$  is close to the average for all cereals together. In India there are about 20 river basins which are presently the source of surface and ground water for many sectors including the irrigation sector. There is a need to work out on the estimation of the water budget components of the basin to go for proper utilization of water resources as water resources and Indian riverine system may face water scarcity situation in near future to come. In this chapter we have discussed the modern tools, techniques and models such as remote sensing, GIS and hydrological models (such as METRIC and SEBAL) which can be used for precise estimation of water budget components in these major river basins.



# **1. INTRODUCTION**

Water is going to be one of the important issues concerning humanity in this century and coming years. The world is already facing a crisis of water supply in terms of both quantity and quality. Water is the most critical requirement of living organisms, and it affects human behaviour in a very significant way. Water is regarded as a gift of nature when available in plenty but becomes precious when its scarcity occurs. Water is also a source of dispute and even conflict between its different stakeholders and users. For 21<sup>st</sup> century, water management is becoming a key issue since growing pressures are negatively affecting the availability and distribution of already scarce fresh water to growing population. There are so many factors behind diminishing water resources such as expanding populations, economic growth, pollution and seasonal climatic conditions. Regular monitoring and forecasting the global water cycle using modern techniques is becoming increasingly important for efficient water management (Ray 2008).

Three major sectors i.e. agriculture, domestic consumption and industry are competiting for water. Globally and in most major river basins, the biggest volumes are withdrawn for irrigation purposes. Water use in agriculture is approximately 70% worldwide. While populations are increasing at a faster rate, the available fresh water resources are diminishing leading to greater scarcity. Precipitation is the major source of fresh water, which is stored temporarily in natural areas or in man-made reservoirs. Around 8% of the annual fresh water renewable resource is used, with 26% of evapotranspiration of water and 54% of runoff (Ray 2008).

#### 1.1. Global fresh water availability and growing pressure

Even though two-thirds of the global surface is covered by water, only 2.5% is fresh water. This fresh water is not evenly distributed across the globe. Freshwater resources are shrinking more rapidly now compared to previous decades due to population explosion. Since 1970, world population has increased by 1.8 billion whereas per capita water availability worldwide is a one third now. From 1970 to 2000, freshwater usage by the agriculture increased by 175% which is consuming 70% of global fresh water. Only an extremely small portion of the 1.36 billion m<sup>3</sup> of global freshwater is available for use. Various complex processes like evaporation and rainfall between the sea and the land surface contribute very small quantities of freshwater, to the tune of 40,000 km<sup>3</sup> annually. Globally, in last 25 years, there is a decrease of 27 percent in per capita water availability; in 1970 it was 10,000 m<sup>3</sup> which declined to 7,300 m<sup>3</sup> in 1995. Countries are regarded as water-stressed when the per capita annual freshwater supply remains between 1,000 and 2,000 m<sup>3</sup> and water scarce when the supply falls below 1,000 m<sup>3</sup>. In India, over the past 50 years, per capita availability of fresh water has declined from 3,000 m<sup>3</sup> to 1,123 m<sup>3</sup>.

Loss of fresh-water due to high evaporation rates is critical in tropical and arid regions. In India, agriculture is mainly dominated by cereal crops such as rice and wheat, and yield of cereals is sensitive to changes in temperature. The projected temperature rise in the twenty-first century, therefore, will have serious implications

243



for India where high evapotranspiration rate and rising temperature, have profound implications for freshwater management policies.

#### 1.2. Fresh water withdrawal in India

Rainfall in India and sub-continental countries (Bhutan, Nepal, Bangladesh, and Pakistan), is limited to three or four months of the year. This monsoon rain also depends on the same riverine systems for freshwater. India currently withdraws a little more than 26% of the available freshwater which is far less than Pakistan, with its rate of 70%, is considered a high water stressed country. Whereas other South Asian countries are using more than 40% of their available water resources. The annual Indian evapotranspiration (ET) rate varies between 1,400 and 1,800 mm. It is highest in west Rajasthan, some parts of Karnataka, Andhra Pradesh, and Tamil Nadu. In some part of country, evaporation some time exceeds 1,800 mm.

With over a billion people, India presently has the world's second largest population and estimate of the population in the year 2050 is 1.7 billion. This is an increase of approximately 50% in population in the coming 50 years. There is a net export of agricultural produces from India, which has shown an increase in the past decade and this trend is likely to continue. These developments will lead to a larger demand in the total food grains production and ultimately more water for the agricultural sector in near future.

### 1.3. Knowledge gap

In most cases data regarding water usage and availability of water is not available which poses a challenge for sustainable management and development of water resources. Hence, measurement and quantification of the energy footprints, water footprints and water balance components is fundamental to understanding the hydrological behaviour of a system for effective water management. In the assessment of water resources and derivation of the water balance, it is important to understand the spatial and temporal dynamics of water footprints, the different components governing the surface energy balance such as evapotranspiration. This information is crucial for planning and development of water resources infrastructure and also agricultural planning. There is a strong need for studying the water and energy balance for integrated water management at river basin scale along with the water footprint of crops. Quantitative evaluation of water resources and their change on the basis of the water balance approach under the influence of human activities are possible if various components of hydrological cycle are studied. Additionally, decision making on water management issues are strengthened by water balance estimates.

Although small scale measurements of components of energy balance and Evapotranspiration (ET) measurements over a crop canopy are done usually by Lysimeters and Eddy Covariance approaches, large spatial scale measurements are still not available. However, estimations of actual ET on large spatial scales would be useful for many water resource applications including estimating agricultural water use and monitoring water rights compliance.



The objective of this chapter is to discuss the water footprints of rice in different cultivation practices in comparison to other crops and discussing the key challenges and issues related to the water management and water balance studies particularly in river basins of India and need for advance methodologies for studying water footprint, and energy balance components.

# 2. WATER FOOTPRINT CONCEPT AND CURRENT SCENARIO

Currently, the ratio of volume of consumptive water use to the quantity of produce of interest which is termed as water footprints may be used to indicate direct and indirect utilization/appropriation of fresh water resources. Both consumptive water uses i.e. the green and blue water footprints and the grey water footprint which is required to assimilate pollution may be used for fresh water appropriations. Lower water footprints from a management system indicate its efficiency to produce more biological yield or product with less amount of water. The water footprint of a product can be used to provide information to consumers about the water-related impacts of products they use or to give policy makers an idea of how much water is being "traded" through imports and exports of the product.

Some major determinants of the magnitude of the water footprint from any area are (Chapagain and Hoekstra 2004):

- the average consumption volume per capita, generally related to gross income
- the consumption pattern of the inhabitants
- climate, in particular evaporative demand
- agricultural practices

#### 2.1. Water footprints in rice production system

There are two major systems of rice cultivation: low wetland and upland cultivation systems. Around 85% of the global rice harvest area is resulting from wetland systems and around 75% of rice production is obtained from irrigated wetland rice (Bouman et al., 2007). In Asia, paddy fields are generally prepared by tillage followed by puddling where the top soil is saturated and water remain stagnated during most of the crop growth period. Whereas, in the USA, Australia and Europe rice fields are dry and flooding is done later.

Chapagain and Hoekstra (2011) made a global assessment of the green, blue and grey water footprint of paddy, using a higher spatial resolution and local data on actual irrigation. They reported that water footprint of rice paddy production globally is 784 km<sup>3</sup> yr<sup>-1</sup> with an average of 1325 m<sup>3</sup>t<sup>-1</sup> (48% green, 44% blue, and 8% grey). They also observed that the ratio of green to blue water varies significantly over time and space. They estimated 1025 m<sup>3</sup>t<sup>-1</sup> of percolation in rice production. They reported that the green water fraction is significantly larger than the blue one in India, Vietnam,

245



Indonesia, Thailand, Myanmar and the Philippines, whereas, in Pakistan and the USA the blue water footprint is four times higher than the green constituent and the virtual water flows which is related to rice trade internationally was  $31 \text{ km}^3 \text{ yr}^1$ . They also reported that rice products consumption in the European nations was accountable for evaporation of 2279 Mm<sup>3</sup> of water and polluted return flows of 178 Mm<sup>3</sup> across the globe, annually mainly in Thailand, India, Pakistan and the USA and the water footprint due to consumption of rice created moderately low stress on the water resources in India as compared to that in Pakistan and the USA.

The calculated mean water depth used in cultivation of rice in each of the 13 major rice-producing countries is presented in Table 1(Chapagain and Hoekstra 2011).

They calculated the total water use  $(m^3 yr^{-1})$  for rice cultivation in each country by multiplying the national harvested area of crop (ha yr<sup>-1</sup>) with the corresponding depth of water (mm yr<sup>-1</sup>) used in rice fields. The water footprint of rice cultivation is thus calculated as the sum of water evaporated from the crop fields and the volume of water polluted in the process (Table 2)

# 2.2. Different Irrigation and tillage methods for reducing the water footprints of rice

Water plays a major role in global agriculture. Due to rising demand of water among various sectors, it is going to be a scarce commodity worldwide. Reduction of crop water footprint is therefore very much essential which can be achieved by lowering the crop water use from the crop fields. Work on improving the water productivity of crop by reducing amount of applied irrigation water, which in turn reduces the crop water footprint through adoption of various irrigation methodologies like alternate wetting and drying, mulching, micro irrigation, namely, drip, sub surface drip and sprinkler irrigation, are going on globally.

S.No.	Countries	Evaporation (green)	Evaporation(blue)	Pollution (grey)
1.	China	228	302	73
2.	India	314	241	34
3.	Indonesia	260	217	53
4.	Bangladesh	192	202	36
5.	Vietnam	139	92	58
6.	Thailand	252	149	31
7.	Myanmar	297	133	18
8.	Japan	219	258	39
9.	Philippines	277	139	26
10.	Brazil	260	220	20
11.	USA	168	618	75
12.	Korea, Rep.	232	253	55
13.	Pakistan	124	699	26

Table 1. Depth of water used in rice production (mm yr <sup>-1</sup> ) for the 13 major rice-						
producing countries for the period 2000–2004.						



	National water footprint of rice production (evaporation + pollution)					
	Green	Blue	Grey	Total		
China	65.2	86.5	20.8	172.5		
India	136.3	104.5	14.7	255.5		
Indonesia	30.3	25.3	6.1	61.7		
Bangladesh	20.4	21.5	3.8	45.7		
Vietnam	10.5	6.9	4.3	21.7		
Thailand	25.2	15.0	3.1	43.3		
Myanmar	19.1	8.5	1.1	288		
Japan	3.7	4.4	0.7	8.8		
Philippines	11.2	5.6	1.0	17.9		
Brazil	8.8	7.4	0.7	16.8		
USA	2.2	8.0	1.0	11.1		
Korea, Rep.	2.4	2.6	0.6	5.6		
Pakistan	2.9	16.3	0.6	19.9		

Table 2. Total national water footprint of rice production and percolation of water in the 13major rice-producing countries (billion m<sup>3</sup> yr<sup>-1</sup>) for the period 2000–2004.

It was found that the moderate alternate wetting and drying technique was able to increase grain yield by 6.1% to 15.2% and water productivity by 27% to 51% at the same time reducing the amount of irrigation water applied by 23.4% to 42.6% when it was compared with conventional irrigation practices (Yang et al. 2017). Alternate wetting and drying of paddy fields under the System of Rice Intensification (SRI) was found to be effective in increasing paddy yield by 78% with about 40% reduction in total amount of applied water for irrigation, which also reduced the costs of production compared to conventional continuous flooding (Sato and Uphoff 2007). Drip irrigation method was found more effective with SRI to minimize water losses and also to increase the rice yield based on field evaluation in India. It has been found that adoption of SRI along with drip irrigation with a dripper spacing of 20 cm with plant to plant spacing of 30 x 30 cm was able to give the highest net return (B:C ratio 3.23) with highest water productivity of 0.90 kg m<sup>-3</sup> and highest water-energy productivity of 7.85 kg kW h<sup>-1</sup> as compared to conventional paddy cultivation (0.16 kg m<sup>-3</sup> and 1.02 kg kW h<sup>-1</sup>, respectively) under continuous flooding (Rao et al. 2017).

A study conducted on winter wheat in Northern China showed that deficit irrigation reduced blue WF (by 38%) with an average yield reduction by 9% and increased irrigation efficiency by 5%. It was also reported that the organic or synthetic mulching practices reduced blue WF by 8% and 17%, respectively with the same yield level with an improvement in water use efficiency by 4% and 10%, respectively. It was also found that under the deficit subsurface drip irrigation (SDI) with organic mulching, irrigation efficiency decreased blue WF by 44% and increased it up to 45% from 36% as was found in case of sprinkler irrigation without mulching.

In a study, consumptive WF of different crops such as potato, maize, and tomato were studied. Data on four irrigation techniques viz. furrow, drip, sprinkler, and

247



subsurface drip (SSD) with four irrigation strategies like full (FI), deficit (DI), supplementary (SI) and no irrigation were analyzed under different mulching treatments. Data from different countries were analyzed. Analysis revealed that water footprint (WF) was found to be reduced to 8–10%, 13%, 17–18%, and 28%, respectively under drip or subsurface drip irrigation, surface drip or subsurface drip with organic mulching, and surface drip or subsurface drip in combination with synthetic mulching, respectively as compared to the control (furrow irrigation, full irrigation, no mulching). They reported that the water footprint of growing a crop was the lowest under drip irrigation, followed by furrow irrigation, sprinkler irrigation system gave the largest consumptive water footprints, followed by furrow irrigation, surface drip irrigation and subsurface drip irrigation. This study interestingly gave the finding that furrow irrigation was able to give less consumptive water footprint of crop as compared to the sprinkler irrigation efficiency of sprinkler irrigation is higher than that of furrow irrigation.

### 2.3. Water footprint in different crops

In the past century the drawing of fresh water has been increased by 7 times due to increase in population pressure and industrialization. At present, the agriculture alone consumes around 85% of global blue water and 99% of global green plus blue water (Hoekstra and Mekonnen 2012). The variation of water footprint was found significant among crops and production regions. Crops having higher yield or biomass generally have a less water footprint as compared to the crops having lower yield and biomass. The mean global water footprint varied largely from sugar crops (197 m<sup>3</sup> t<sup>\*1</sup>), vegetables (322 m<sup>3</sup> t<sup>\*1</sup>), fodders (253 m<sup>3</sup> t<sup>\*1</sup>), roots and tubers (387 m<sup>3</sup> t<sup>\*1</sup>), fruits (967 m<sup>3</sup> t<sup>\*1</sup>), cereals (1644 m<sup>3</sup> t<sup>\*1</sup>), oil seed crops (2364 m<sup>3</sup> t<sup>\*1</sup>) and pulses (4055 m<sup>3</sup> t<sup>\*1</sup>). The mean water footprint of creeals is about 1644 m<sup>3</sup> t<sup>\*1</sup>. Among them, the water footprint for millet is comparatively large (4478 m<sup>3</sup> t<sup>\*1</sup>), while for maize it is comparatively small (1222 m<sup>3</sup> t<sup>\*1</sup>). The mean water footprint of rice (1673 m<sup>3</sup> t<sup>\*1</sup>) is close to the average for all cereals together (Fig.1). Crops like soybean, sorghum and cotton has larger water footprint than rice (Mekonnen and Hoekstra 2013).

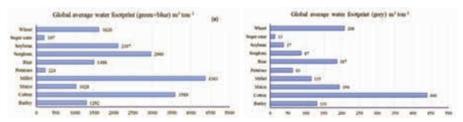


Fig. 1. Global average water footprint (m<sup>3</sup> ton<sup>-1</sup>) (a) green+blue (b) grey (modified after Mekonnen and Hoekstra 2014).

Kar et al. (2014) has analyzed the water foot prints for maize crops, irrigated rice, rainfed rice and water footprints of some winter crops (maize, groundnut, sunflower,



wheat, potato) grown in rice fallow in Odisha. They reported a higher yield by 59%, 29 %, 33-%, 58-%, and 19% in respective crops when three irrigations was applied and with four supplemental irrigation there was an increase of yield by 214%, 89%, 78%, 81% and 54%, respectively, for maize, groundnut, sunflower, wheat and potato over two irrigation. They also reported that water footprints of the crops were less when there was an increase in the crop yield at higher irrigation levels. They also estimated the water footprints of crops like lathyrus, black gram, pea and chickpea under various seeding or tillage methods in rainfed lowland rice fallow like relay (farmers' practice), one ploughing and sowing on same day, two ploughing in different days and sowing after second ploughing, zero tillage treatment, two ploughing in different days and sowing after second ploughing (5941, 6754, 6678, 8500 m<sup>3</sup>ton<sup>-1</sup> for lathyrus, black gram, pea and chick pea, respectively), whereas the water footprint was maximum under farmers' practice (298, 8657, 12833, 9850 and 15455 m<sup>3</sup>ton<sup>-1</sup> for lathyrus, black gram, pea and chick pea, respectively).

# 3. WAYS TO MINIMIZE THE WATER FOOTPRINTS UNDER DIFFERENT CROPPING SYSTEM

The crop evapotranspiration and thereby water foot prints in various crop production system can be minimized by minimizing irrigation water losses through adoption of modern scientific irrigation approaches like adoption of micro-irrigation technology, adoption of conservation agriculture like SRI technique of rice cultivation and mulching, precision land leveling techniques for uniform water application in field, and optimal use of fertilizer in fields in order to reduce the grey water foot print.

# 4. THE PROJECTED FUTURE WATER DEMAND FOR MAJOR RIVER BASINS OF INDIA

Different river basins have their reach in different states of India. Water demand in industrial and domestic sectors besides the irrigation sector is on a rising trend day by day. River basins are mainly the sources of water for all those sectors. Due to rising water demands from various sectors many river basins are going to be water scarce by 2050. It has been projected that several basins in India would deplete more than 60% of potentially utilizable water resources that will be available in 2050, and face acute water scarcities. Some of the major river basins like Indus and Ganga, will have as high as 0.07181 billion m<sup>3</sup> and 0.02425 billion m<sup>3</sup> deficit water supply for industrial and other sectors by 2050, whereas river basin like Mahanadi will be able to supply 20% and 33% less water as compared to water available in 2010 for industrial and other sectors due to a rising demand of water amongst various sectors (Gaur et al. 2011). This serious threat of water scarcity from various Indian river basins must be dealt with priority.



In India there are about 20 river basins which are presently the source of surface and ground water for many sectors including the irrigation sector. Among surface and ground water, the availability of surface water is highly seasonal, whereas the groundwater is a steady source of water throughout the year. In terms of water availability from the river basins which comprises both the surface water and the groundwater, groundwater constitutes on an average 50%, though inequalities exists across river basins. As per estimation from the Ganga river basin, the share of groundwater in the total water storage is about 64%, whereas, from basins like Krishna, Mahanadi, Subernarekha, and Narmada it is about 35% or less. At present irrigation sector in India is the most consumer of water from most of the river basins from India. Due to rise in population the water demands in industrial and domestic sectors besides the irrigation sector is increasing rapidly due to which many of the Indian river basins are becoming water scarce. It is going to be more serious in 2050 when Ganga and other river basins like Mahanadi and Pennar will also face severe water availability problems (Gaur et al. 2011). Estimating the water balance components on river basin scale will help in taking best water management measures especially in irrigation sector to combat the water scarcity situation at basin level.

# 5. EVAPOTRANSPIRATION IN DIFFERENT RIVER BASINS

Evapotranspiration (ET) percentage for Narmada, Godavari, Cauvery and Krishna basins, varies between 48.5 and 59.8% of precipitation. For these basins, overall ET value is 58.3%. For the trans-boundary basins, such as Indus, Ganga and Brahmaputra, ET is 23.1% of precipitation for Ganga and 71.7% for Indus basins. For these basins, overall ET is 17.6%.

#### 5.1. Water balance studies: Current status and future scenario

Based on hydrological simulation of Mahanadi River Basin and impact assessment of Land Use and Land Cover (LULC) change on surface runoff generation through use of hydrological model, an increased pattern in the annual flow of stream by 4.53% was found in the Mahanadi river basin which was contributed to the reduction in forest cover by 5.71% (Dadhwal et al. 2010). Water budget components estimation on a point scale using basic water budgeting equation with the help of GIS was done in the Lower Yamuna Basin, Delhi. Based on water balance estimation, it was found in the study that all stations in the region are dry as the annual rainfall in the region remains short of annual potential evapotranspirative demands (Ahlawat 2014).

Water resource assessment of Narmada Basin, India was done by using the Variable Infiltration Capacity (VIC) hydrological model. It was observed that there was a substantial increase in evaporation component by 0.56% whereas with a decrease in runoff, base flow and stream discharge by 42.42%, 34.18% and 34%, respectively in year 2005 in comparison with year 1975 due to change in LULC because of construction of Indira Sagar Dam during the analysis period (Shiradhonkar 2015).

The water balance components of Chambal river basin using VIC model was estimated. It was observed that the land use and land cover, soil and slope

250



characteristics were the main parameters influencing the hydrological processes in considerable manner. The annual runoff over the basin was 50% with a higher runoff from areas having less vegetation, higher slopes. The runoff was also found to be affected in considerable manner with respect to soil type and soil characteristics over the area.

Impact study of Climate Change on the Hydrology of Mahanadi river basin was done using Statistical Downscaling Model (SDSM). A decrease in precipitation pattern for the time period 2020s and 2080s annually and seasonally was found from downscaling of the precipitation in future scenarios through use of SDSM (Pandey 2015).

Impact of climate change on water balance in Krishna river basin was under taken where the water balance components were estimated using semi-distributed hydrological model namely Soil and Water Assessment Tool (SWAT). Based on climate projections estimated that in the period 2041-70 (2050s) there will be increase in the annual precipitation, surface runoff, water yield and actual evapotranspiration as compared to the baseline simulation period (1961-1990) whereas no substantial change for these parameters were observed by the model runs in 2020s (2011-2040).

The runoff, sediment and water balance components of Ken basin, India, were estimated using remote sensing derived products (SRTM DEM), gridded precipitation and temperature data (LANDSAT TM data), and using Soil and Water Assessment Tool (SWAT) within a geographic information system (GIS) modeling environment. It was found that evapotranspiration was more predominant which was around 44.6% of the average annual rainfall falling over the area whereas the stream runoff was 34.7% and deep aquifer recharge is 19.5% for the river basin (Himanshu et al. 2017).

#### 5.2. Estimating the evapotranspiration

For studying the water balance over a river basin, the most important, challenging and variable component is the Evapotranspiration (ET) over the river basin.

Quite a few methods are used to measure or estimate ET, including hydrological approach, micrometeorological method and plant physiological approaches. Eddy covariance (EC) is the only direct and accurate measurement method which provides latent heat flux (LE) and sensible heat flux (H) as independent variables. Globally though works in this field has been done by researchers but literature availability on works related to eddy covariance method from India are rare. Eddy covariance method was used in Philippines for estimating the actual ET in direct-seeded rice field and it was observed that the average growing season ET rate varied from 4.13 to 4.36 mm d<sup>-1</sup> in 2011 and 2012, respectively. They observed ET of growing rice in the range of 400–556 mm in Philippines. Timm et al. (2014) in Brazil reported that ET reached almost 7 mm d<sup>-1</sup> at the end of the reproductive phase (flowering) of rice crop when leaf area index was at its peak (4.57 m<sup>2</sup> m<sup>-2</sup>). Hatala et al. (2012), using the eddy covariance method, estimated daily evaporation up to 10 mm d<sup>-1</sup> in a rice paddy field. In India, Tyagi et al. (2000) reported an ET of 587mm in rice through eddy covariance method, and the same was found to be 701mmthrough water balance approach.



#### 5.3. ET measurements over spatial scales

Because it involves the transfer of large quantities of water away from earth's surface, the combined effects of evaporation from soil and leaves and transpiration from plants can have important implications for water resources. Small scale measurements of evapotranspiration (ET) are already prevalent and well-established. Eddy-covariance stations, for example, are frequently used to determine turbulent fluxes, representative of a relatively small surface area, up to hundreds of meters. Unfortunately, these measurements cannot be easily extrapolated over larger landscapes due to heterogeneities in land characteristics such as elevation, vegetation, and soil types (Choi et al. 2009).

A number of algorithms utilizing remote sensing to retrieve ET on a large scale have been developed. In addition to providing estimates for larger scales than in situ measurements, remote sensing is often inexpensive for the user to implement as many of the satellite platforms are developed by the government and data are freely available to the public. Two such operational ET modeling schemes include METRIC (Mapping Evapo Transpiration at high Resolution with Internalized Calibration) and the Fusion scheme made up of ALEXI (Atmosphere-Land EXchange Inverse model), DisALEXI (Disaggregated ALEXI) and STARFM (Spatial and Temporal Adaptive Reflectance Fusion Model). The basis for both the METRIC and Fusion modeling schemes is the surface energy balance.

# 6. ENERGY BALANCE OVER RIVER BASINS COVERING RICE CULTIVATION AREAS

Agro-ecosystem productivity rapidly responds to all the climatic variables like atmospheric temperature, precipitation, humidity, solar radiation, and photosynthetically active radiation (PAR). The formation of clouds and succeeding precipitation is dependent on the heat fluxes which are governed by incoming and outgoing radiations. The dynamics of heat fluxes are determined by the nature and type of vegetation covering the soil. Therefore, the determination of a correct energy balance (EB) mechanism is a crucial prerequisite to understand and model an agroecosystem and its interaction with the climatic variables, which is associated with the yield of the crop. Energy and mass transfer are two most important biophysical processes that influence the EB in an agroecosystem. The lowland river basins are mainly favoured for lowland rice and rice based cropping in eastern India. The lowland rice has a unique characteristic, since it grows under semi-aquatic environment or flooded environment. Such environment differs greatly from other upland based crop ecosystem since a continuous water layer is maintained above the soil surface which strongly influenced the surface EB components. Therefore, the exchange of carbon dioxide, methane, water vapour and energy in flooded rice ecology varies to a great extent and shows a close interrelationship between carbon cycle, hydrological cycle and energy balance. Such differential nature of rice cultivation may modify the surface runoff, groundwater storage, water cycle, surface energy budget and possibly the microclimate of the region.

252



Surface EB is mainly described by four types of energy fluxes, i.e. net radiation flux (Rn), sensible heat flux (H), latent heat flux (LE), and soil heat flux (G) approaching into or going out of the soil or water medium. The H is directed away from the surface throughout the daytime, while it is in opposite direction during the evening and nighttime. The LE is the result of evaporation and evapotranspiration at the surface. The Rn is a consequence of radiation balance at the surface, a resultant effect of upwell and downwell radiations. During the daytime, solar radiation is usually dominated and Rn is directed towards the surface of the soil, while vice-versa at nighttime. The G at the surface of soil was dissimilar with the soil beneath after certain depth, and the G at the surface achieved better closure.

#### 6.1. Land characteristics influencing energy balance

The nature of soil and land plays an important role in the EB by influencing energy flux in the soil profile. These influences determine the change in soil temperature in the soil profile, which ultimately control microclimate of the crop-soil-water continuum. The thermal characteristic of soil varies with soil water content, maximum and minimum air temperature, porosity, vapour pressure, saturated vapour pressure and water vapour content. The ground surface gets heated more during the day by insolation than layers underneath, resulting in temperature gradient between the surface and subsoil on the one hand and surface and air layers near the ground on the other. Within the soil this causes heat flow downward as a thermal wave, the amplitude of which changes with depth. Estimation of soil heat flux (G) from the soil temperature data can provide an understanding of the gain or loss of heat by the soil from the atmosphere. The Bowen ratio method is an indirect method that has been widely applied and tested in various environments to characterize the land. This ratio describes the relationship between sensible (H) and latent heat (LE) fluxes and can be used as a measure of evapotranspiration including tall vegetation. Surface albedo is another important land characteristic which determines the surface energy budget and inuences the distribution of radiation energy in earth-atmosphere system and further regulates the atmospheric circulation patterns and hydrologic processes. It strongly depends on soil moisture and temperature (Zheng et al. 2014). Surface emissivity is a measure of the efficiency with which surfaces convert kinetic into radiant energy.

#### 6.2. Estimating energy balance using a single eddy covariance system

A field experiment was conducted using a single eddy covariance (EC) system to study the surface energy budget and energy balance closure (EBC) in rice-rice ecology at ICAR-National Rice Research Institute, Cuttack (Unpublished). Due to the presence of standing water in the rice field, the average latent heat flux at surface and canopy height was higher than sensible heat flux at surface and canopy height, respectively. The average value of residual heat flux (R) was 10.3-12.0% higher in wet season compared to dry season. Soil temperature (Tg) was highest in dry fallow, while the skin temperature (Ts) was highest in dry season. Average Bowen ratio (B) ranged from 0.21-0.60 and large variation in B was observed during the fallow periods as





compared to the cropping seasons. The magnitude of aerodynamic, canopy and climatological resistances increased with progress of cropping season was found smallest during the fallow period. The actual evapotranspiration  $(ET_a)$  measured during both the cropping seasons was higher than the fallow period.

#### 6.3. Remote sensing for energy balance studies

Satellite technologies are now helpful in quantifying the radiation and energy exchanges between sun, earth, and space. Nevertheless, satellites cannot directly measure the magnitude of the energy flows within the atmosphere and at the Earth's surface. Evapotranspiration is the most difficult hydrological flux to estimate or model especially at regional or global scales for assessing the water resource base. Evapotranspiration estimation based on weighing lysimeter, Energy Balance Bowen Ratio (EBBR), eddy covariance techniques, pan-measurement, sap flow, scintillometer, etc. are mainly based on complex models and equations. Moreover, these methods are variable for local, field, and regional scales. These conventional methods can provide the accurate estimates of ET over a homogeneous area. But natural heterogeneity of the land surface and complexity of hydrologic processes do not favor upscaling of such measurements. Remotely sensed images are a promising source of data for mapping regional- and meso-scale patterns of ET on the Earth's surface. Remote sensing images with visible, nearinfrared, and thermal infrared bands are used to retrieve the land surface temperature. These surface parameters estimated from satellite data are used for simulating surface fluxes and ET.

# 6.4. Advantages of space technologies for energy and water balance studies

Remote sensing data presents an interesting opportunity as it allows for the quantification of key water balance components such as evapotranspiration. Space technology applications have begun to permeate many aspects of life in our modern societies. A growing number of activities–weather forecasting, global communications and broadcasting, disaster prevention and relief–increasingly depend on the unobtrusive utilisation of these technologies. The main advantages of using satellites are summarised by Payne et al. (2006). Satellite data may be collected year-round and can provide information when field data collection is not possible, due to remote locations or bad weather conditions. This method also reduces cost when compared to traditional field data collection methods in remote environments (landcover classification for example). Remote sensing may be an option for supplementing more intensive sampling efforts and help extrapolate findings. It is also possible to measure elements of the global water cycle using diverse space-based systems. The estimated residence time for water range from one week (*e.g.* biospheric water) to 10,000 years (e.g. ground water) – hence the need for reactive, timely and long-term observations.

#### 6.5. Limitations of remote sensing for energy balance studies

Uncertainties in the radiance measurement caused by atmosphere require the corrections for the atmospheric effects. There is requirement of increasing accuracy

254

of some land surface variables derived from remote sensing images for increasing the accuracy of ET estimation. It's very difficult to estimate the surface parameters such as surface temperature from heterogeneous surfaces compared to homogeneous well-watered vegetative surfaces. Differences in received radiances will occur due to the differing amounts of soil and vegetation in the field of view when sensor viewing changes from one angle to another.

Different models are used for different land surface characteristics. However, till date, there is no universal model, which could be used throughout the world irrespective of the changes in land surface characteristics, in the climate and terrain without any modification or improvement to estimate the ET from satellite data. Meteorological data which are collected at near-surface height required in most of the ET models. These meteorological parameters are estimated at a satellite pixel interpolation. Accuracy of the interpolation methods are required to be improved while using the data for different climate and terrain conditions.

Nocturnal transpiration and dew may also affect significantly the ET estimation. Nocturnal transpiration has been widely observed using sap-flow and gas exchange measurements with ratios of night-time to day-time transpiration as large as 25% being reported (Dawson et al. 2007). If nocturnal transpiration occurs at sites with high LAI, this process could be an important source of error in remote sensing based ET estimation because of its association with nocturnal vapor pressure difference and wind speed. Adversely, at sites with low LAI, this process will tend to reduce this source of error so that it may be ignored when considering daily TIR-based estimates of ET.

# 7. WAY AHEAD AND FUTURE WORK SCOPE

To check the decline of surface and ground water resources in India it is highly essential to adopt suitable agricultural techniques and agricultural management practices for yield maximisation with less consumption of water. At present there is a need for prioritising the appropriation of fresh water resources to different components or crop production system, domestic and industrial sector to produce a particular product or to complete one process requiring water from a particular management system. The appropriation of water among different sectors or different crop production systems is very difficult unless there are some sound methods or indices for quantifying the water requirement from this production system.

There is a need to work out on the estimation of the water budget components of the basin to go for proper utilization of water resources as water resources and Indian riverine system may face water scarcity situation in near future to come. Remote sensing, GIS and hydrological models are the modern tools that can be used for precise estimation of water budget components in these river basins. As part of the National Communication (NATCOM) project by the Ministry of Environment and Forests, impact of the climate change on the water resources of Indian river systems was quantified and the initial analysis revealed severity of droughts and intensity of



floods in various parts of the country may get deteriorated. Governments must increasingly ensure that farmers use water resources efficiently, and that they are allocated among competing demands in a way that enables farmers to produce food and fibre, minimise pollution and support ecosystems, while meeting social aspirations. Also, the government should ensure the internal water-sharing issues based on sound, internationally accepted principles. A failure to do so would create internal water-related conflicts.

#### References

- Ahlawat R (2014) Pixel-wise surface water balance computation: Lower Yamuna basin. International Journal of Advanced Remote Sensing and GIS3(1):627-641.
- Binayak R (2008) Water: The looming crisis in India. Lexington Books. ISBN-13: 978-0-7391-2601-1.
- Bouman BAM, Lampayan RM andTuong TP (2007) Water management in irrigated rice: coping with water scarcity. Los Baños (Philippines): International Rice Research Institute. p. 54.
- Chapagain AK and Hoekstra AY (2011) The blue, green and grey water footprint of rice fromproduction and consumption perspectives. Ecological Economics 70:749–758.
- Dawson TE, Burgess SSO, Tu KP, Oliveira RS, Santiago LS and Fisher JB (2007) Nighttime transpiration in woody plants from contrasting ecosystems. Tree Physiology 27:561– 575.
- Gaur A and Amerasinghe P (2011) A river basin perspective of water resources and challenges. India Infrastructure Report.
- Hatala JA, Detto M, Sonnentag O, Deverel SJ, Verfaillie J and Baldocchi DD (2012) Greenhouse gas (CO<sub>2</sub>, CH<sub>4</sub>, H<sub>2</sub>O) fluxes from drained and flooded agricultural peatlands in the Sacramento–San Joaquin Delta. Agriculture Ecosystemand Environment 150:1–18.
- Himanshu SK, Pandey A and Shrestha P (2017) Application of SWAT in an Indian river basin for modeling runoff, sediment and water balance. Environmental Earth Sciences 76(1):3.
- Hoekstra AY and Mekonnen MM (2012) The water footprint of humanity. Proceedings of the National Academy of Sciences 109(9):3232-3237.
- Hoekstra AY, Chapagain AK, Aldaya MM and Mekonnen MM (2011) The water footprint assessment manual. Setting the Global Standard1- 224.
- Kar G, Singh R, Kumar A and Sikka AK (2014) Farm level water footprints of crop production. Bulletin No 67. ICAR-Directorate of Water Management, Bhubaneswar, p. 56.
- Mekonnen MM and Hoekstra AY (2013) Water footprint benchmarks for crop production, Value of Water Research Report Series No. 64, UNESCO-IHE, Delft, the Netherlands.
- Pandey P(2015) Impact of climate change on the hydrology of Mahanadi river basin, M.Techdissertation- (http://ethesis.nitrkl.ac.in/6676/1/Pooja\_M.Tech\_(R)\_Thesis.pdf.; Accessed on 20<sup>th</sup> October, 2017).
- Payne JF, Shuchman RA, Josberger EG, Liversedge L and Noyles C(2006) Remote sensing of the Bering glacier region, 9th Bi-Annual Circumpolar Remote Sensing Symposium, 15-18 May.
- Rao KVR, Gangwar S, Keshri R, Chourasia L,Bajpai A and Soni K (2017) Effects of drip irrigation system for enhancing rice (*Oryzasativa* L.) yield under system of rice intensificationmanagement. Applied Ecology and Environmental Research, 15(4):487-495.



- Sato S and Uphoff N (2007)A review of onfarmevaluations of system of riceintensification(SRI) method in easternIndonesia. In: Perspectives in Agriculture, Veterinary Science, Nutrition and NaturalResources. Commonwealth AgriculturalBureau International, Wallingford, UK.
- Shiradhonkar SR(2015) Water resource assessment at a basin level using water balance approach and remote sensing inputs. M.Tech Thesis (http://www.iirs.gov.in/iirs/sites/default/files/ StudentThesis/SAKSHI\_MTech\_2013-15.pdf; Accessed on 15th October, 2017).
- Timm AU, Débora RR, Nereu AS, Luis GGG, Otávio CA, Osvaldo LLM, Virnei SM, Gervásio AD, Mitja F and David LT(2014) Energy partitioning and evapotranspiration over a rice paddy in southern Brazil. Journal of Hydrometeorology 15(5):1975–1988.
- Yang J, Zhou Q and Zhang J (2017) Moderate wetting and drying increases rice yield and reduces water use, grain arsenic level, and methane emission. The Crop Journal5(2):151-158.
- Zheng Z, WeiZ, Li Z, Wei H and Liu H (2014) Study of parameterization of surface albedo of bare soil over the Gobi desert in the Dunhuang region. Chinese Journal of Atmospheric Sciences 38:297–308. <sup>⊕</sup>



# Agro Ecological Intensification of Rice-based Cropping System

## BB Panda, BS Satapathy, AK Nayak, R Tripathi, M Shahid, S Mohanty, D Bhaduri, R Khanam and PK Nayak

# SUMMARY

Agroecological intensification of rice based cropping system mainly aims at achieving maximum system productivity with minimum environmental impact by managing and organizing crops in a way that they best utilize the available resources (soil, air, sunlight, water, labour, equipments) and beneficial interactions among themselves. Based on agro-ecological conditions, market and domestic necessities and facilities available with farmers, the dominant rice based cropping systems followed in eastern India are rice-rice, rice-wheat, rice-rapeseed/mustard, ricegroundnut, rice-potato and rice-pulses etc. Cropping system research systematically focused on development and management of site specific suitable rice based cropping system particularly for rainfed regions of the country to ensure livelihood security of the poor, small and marginal farmers. Ecological intensification is conceptualized to increase crop yields per unit land, time, and other consumable resources used in food production. Therefore, for improving the profitability, addressing biodiversity conservation, climate change mitigation and long term sustainability we have to exploit the food production system through ecological intensification of suitable rice based cropping systems.

### 1. INTRODUCTION

Indian agriculture is largely dependent on rice based cropping systems. Productivity enhancement of these systems has been proved as an important means of achieving livelihood security, higher income, reduced poverty and employment generation. Rice-based cropping system secures the livelihood of more than half a billion farm families. Since Green revolution, the food grain production has increased manifold in India with adoption of input responsive high yielding varieties. However, urbanization and globalization and rapid economic growth has already brought a considerable change in the food habits from staple food towards fruit, vegetables etc. Hence, the per capita rice consumption has followed a declining trend and it seems the trend would continue till 2030 indicating for an urgent attention toward diversified and intensified cropping system. Adding to it cereal based cropping systems has raised many uncommon issues now and continuation of the green revolution practices has degraded the soil health and fertility, brought dominance of weed flora, soil pathogen etc.

Rice-based cropping systems also produce and emit dominant green house gas (GHG) methane that largely controlled by water and residue management practices.

Further, the importance of prevailing rice based cropping system is now-a-days losing ground due to decrease in factor productivity. Therefore, identification of suitable rice-based cropping systems with higher resource use efficiency fitting to the local agro ecological situation is the need of hour for improving the profitability and employment generation in agriculture.

Diversification has been well reported as an alternative option for sustaining the land productivity and enhancing farm income for achieving a stable agricultural development. In eastern Indian states, rice-based cropping systems are the most dominant system covering 43% of the country's rice growing area. Rice-based systems are intimately connected with development of water resources as rice is a big consumer of water.

However, the natural resources are shrinking day by day making resource-use efficiency an important issue while considering the suitability of any cropping system. As rice is synonymous with life of eastern Indian people any alternation to the existing system with a tendency to decline rice productivity will be neither sustainable nor acceptable to the farmers. Therefore, component crop selection to be well maneuvered to harvest the synergism among this crops for efficient utilization of resources while mitigating the adverse environmental impacts.

In this chapter authors have tried to identify the issues associated with rice based cropping system. Current station of diversification and intensification of the rice based cropping system has been discussed. Further the optimal management options indentified in different parts of the country and abroad has been explained. The problem and management options for addressing rice fallows particularly in eastern India has been summarized.

### 2. Agro-ecological intensification

Agricultural intensification aims at increasing the productivity of existing land and water resources in the production of agriculture and allied products. Intensification was generally associated with higher external inputs use earlier but now defined as more efficient use of production inputs. Use of high yielding varieties with higher resource/labour use efficiency resulted substantial productivity enhancement. Better management practices has already brought in rice intensification (SRI) in most of the rice growing countries raising the land, water, seed and labour productivity (Hassan et al. 2015). However, ecological intensification (EI) is gaining momentum now internationally. Ecological intensification is conceptualized as an increasing crop yields per unit land, time, and other consumable resources used in food production. It is particularly relevant for addressing biodiversity conservation and climate change mitigation (Laurance et al. 2014). The original vision of ecological intensification was based on narrowing the yield gap, soil quality improvement and precise application of inputs (Cassman 1999).

An estimate says that India is home to more than 250 cropping systems. Based on rationale of spread of crops; about 30 cropping systems have been identified as important cropping systems. Among these, rice-based cropping system is the major



cropping system practiced in India. In rice ecosystem of eastern India, rice-rice, ricewheat, rice-rapeseed/mustard, rice-groundnut, rice-potato and rice-pulses are commonly practiced cropping sequences. With the introduction of stress tolerant and short duration varieties, irrigation facilities and conservation practices there is tremendous scope for crop intensification for increasing system productivity and income of farmers. Diversification of crops and cropping systems having higher water productivity, economic profitability and long-term sustainability with the availability of modern management techniques may prove a better option in this respect. Inclusion of legumes and oilseeds using intensification approach based on resource availability can make a substantial enhancement in productivity and income in addition to soil fertility improvement.

Rice based cropping system seems to be continued as the mainstay of Indian agriculture. To achieve the livelihood security of the poor, small and marginal farmers, we need to develop rice based system with higher productivity and profitability by practising multiple cropping both in irrigated and rainfed ecologies. A number of alternative cropping systems have already been identified (Table 1) for different regions of the country and neighbouring countries. It is necessary to enhance the profitability and sustainability of newly developed diversified and intensified cropping system through appropriate management practices. Simply accelerating yield gains of existing food production system leads to amplification of environmental degradation which is not sustainable for ensuring food security. Therefore, we have to exploit the food production system through ecological intensification of existing cropping systems.

#### 2.1. Rice-wheat cropping system

Rice-wheat cropping sequence is the predominant cropping system covering about 10.5 million hectares of area in the country with a major contribution to the cereal production. Continuous adoption of rice-wheat sequence has led to weed flora shift; disease and insect-pest build up, decline in factor-productivity, which ultimately resulted in declining the system efficiency and productivity. Hence rice-wheat system can be made sustainable and profitable following the ecological approaches. For example, rice-vegetable pea-wheat-greengram sequence was a better system than rice-wheat at Karnal, Harvana, whereas hybrid rice-gobhi sarson-okra system registered maximum water productivity, production efficiency and rice equivalent yield compared to the pre-dominant cropping system of the region i.e. rice-wheat and rice-chickpea in clay loam soils of Jabalpur (MP). Desai et al. (2016) concluded that resource rich farmers can diversify the existing rice- wheat- fallow system to rice-sorghum-sorghum ratoon system for obtaining higher system productivity and profitability in south Gujarat but resource poor farmers can adopt rice-sweet corn- black gram or rice-green gram-groundnut cropping system for improving the soil fertility. In Punjab, maizepotato-onion cropping system has been recommended as an efficient cropping system for replacing existing rice-wheat system. Similarly, rice-wheat cropping system can effectively be replaced with rice-vegetable systems with the inclusion of vegetables like french bean, potato, cauliflower, rajmash, peas and garlic to fulfil the daily needs of farmers and to get higher returns and better soil health. The rice-chickpea-vegetable



cowpea cropping system can be suggested for diversifying rice-wheat system in the irrigated situations of Kumaon Himalayas.

Intercropping of legumes, vegetables and other high value crops along with the new crop establishment techniques i.e. FIRBS is the way towards farmer's nutritional security and economic growth (Naresh et al. 2017). Prasad et al. (2013) observed that intercropping of potato with wheat and inclusion of greeengram seems more productive, resource-use efficient and remunerative compared to rice—wheat or rice—fallow systems under irrigated ecosystem of Jharkhand. Saha et al. (2010) reported that rice-potato-blackgram and rice-maize (cob) + vegetable pea (1:2)-green gram cropping system was superior to rice-wheat system in sandy clay loam soils of Varanasi, UP.

Introduction of green manuring or leguminous crops in the existing rice-wheat system in our neighbouring country Pakistan not only increased grain yields but also improved the physio-chemical properties, organic matter contents and nutrients availability. Unlike Pakistan, additions of cereal crops under cereal based cropping system were also found most energy, environmentally and economically efficient cropping systems for tropical region of Nepal (Pokhrel and Soni 2017).

#### 2.2. Rice-rice cropping system

Next to rice-wheat, rice-rice is another extensively cultivated cropping system spreading over 6 million hectares area in India. Poor resource use efficiency, micro nutrient deficiency and deterioration of soil physical properties are major issues for sustaining productivity of rice-rice cropping system. Sharma et al. (2016) suggested for adoption of good agricultural practices and conservation agriculture for increasing the productivity and income of rice based cropping systems. Baisya et al. (2016) revealed that highest rice-equivalent yield and nutrient-use efficiency and productivity can be achieved by cultivation of cabbage-greengram/blackgram in place of summer rice. Under irrigated lands of Bihar, rice-tomato-bottle gourd, rice-potato-onion, rice-coriander–lady's fingers, rice–carrot–cowpea and rice–mustard–tomato cropping system was most productive with rice-equivalent yield of (27.04 t ha<sup>-1</sup> year<sup>-1</sup>), production efficiency (83.97 kg ha<sup>-1</sup> day<sup>-1</sup>), profitability (Rs. 320.36 ha<sup>-1</sup> day<sup>-1</sup>), and higher net return of Rs. 1,16,929 ha<sup>-1</sup> year<sup>-1</sup> as compared to other cropping systems (Prasad 2016).

In rice-rice double cropped areas of Krishna–Godavari delta of Andhra Pradesh, replacing dry season rice crop with maize recorded higher system productivity along with economic efficiency whereas rice–mustard system recorded highest system energy use efficiency (Rao et al. 2014). Sravan et al. (2014) observed that inclusion of sunnhemp and blackgram is the best system for North Coastal Zone of Andhra Pradesh. In the high rainfall zone of south Gujarat rice–fenugreek–okra system was most productive and remunerative followed by the rice–onion–cowpea system.

Inclusion of high yielding mustard either under tilled or zero tilled relay cropping can also bring substantial improvement of rice- rice cropping system (Hassan



et al. 2015). Alam et al. (2017) opined that diversifying *boro* rice with wheat-mungbean is economically superior to *boro* rice-*aman* rice system. A additional crop of pea as green pod vegetable in rice–fallow–spring irrigated rice cropping system increased farm productivity by 1·4-fold and income by four fold (Malik et al. 2017). However, in non-saline areas of Bangladesh, the most profitable cropping pattern is rice -mustard-jute and rice-potato-jute compared to rice-boro rice cropping system. Further intensification of the double rice cropping sequence with addition of mustard, mungbean in lowland rice ecosystem of Bangladesh can be done for higher productivity, soil enrichment, economic benefit and employment.

In irrigated medium land of coastal Odisha rice-sunflower-greengram, rice-maizecowpea, rice-potato-sesame are found promising with highest rice equivalent yield of 18.8 t ha<sup>-1</sup> which was recorded in rice-potato-sesame cropping sequence followed by rice-maize-cowpea (18.2 t ha<sup>-1</sup>). Based on the research work done under different onfarm trials in rice based cropping sequence, watermelon, chilli and sunflower found promising crops for both medium and high salinity areas whereas, lady's finger found most remunerative under medium salinity condition after rice.

#### 2.3. Rice-maize cropping system

Rice-maize cropping system are practised about in 3.5 million hectares areas in Asia while it is grown in 0.53 million hectares in India. This system is developing very first in Bangladesh, South and North India. Rao et al. (2014) reported that shifting rice-rice double cropping in Krishna-Godavari delta areas of Andhra Pradesh to rice-maize, recorded higher system productivity and economic efficiency whereas higher system energy use efficiency was observed in rice-mustard and higher land use efficiency was recorded in groundnut. Rice-rice or rice-fodder maize sequence had an adverse impact on yield sustainability as it decreased the yield in low land rice ecosystem of Karnataka but rice-soybean cropping sequence maintained/sustained the rice yield on long term basis. Bastia et al. (2006) has concluded that rice-maize-cowpea was the most productive and remunerative cropping system in Bhubaneswar, Odisha.

#### 2.4. Rice-pulse cropping system

Rice-pulse cropping system are dominant crop rotation in eastern India particularly in Chhattisgarh, Odisha and parts of Bihar. Rice-blackgram, rice-greengram sequence or as *paira* crop are popular and unique in rainfed lowland rice ecosystem of Odisha, where these two tropical crops are grown in winter season after rice and it is subjected to low temperature. Dwivedi et al. (2016) proved that rice-rice ratoon-greengram sequence was best combination in terms of production and energy efficiency in coastal Odisha. Growing of jute-rice-greengram offers more advantages than the traditional cropping system as this system of crop intensification could improve soil fertility and increase crop productivity. Sunnhemp-rice-black gram has also registered the highest returns per rupee invested (2.52) and hence was suggested for Coastal Andhra Pradesh.



#### 2.5. Rice-oilseed cropping system

Rice-oilseeds cropping sequences are considered as valuable cropping system for food and nutritional security. In eastern India, rice-groundnut, rice-rapeseed/ mustard are predominant cropping systems. The rice-groundnut cropping system can be replaced with rice-brinjal cropping sequence in mid-central table land zone of Odisha as it provides maximum net return owing to higher system productivity (Samant 2015). The finding by Lal et al. (2017) at NRRI, Cuttack demonstrated that early sowing of dry season toria after rice is profitable and it ensures profitability of rainfed rice based cropping system.

#### 2.6. Rice-fallow

Rice fallow includes medium and low lands, which are kept fallow after *kharif* paddy. The prevailing practice of leaving land fallow after harvest of rice is no more economical and sustainable. Odisha accounts for around 10% (12.2 lakh ha) of the total rice fallow of India (11.65 m ha). Utilization of fallow lands is likely to generate considerable income and employment opportunities for the millions of small landholders in the region. Efficient and proper utilization of rice-fallow lands in dry season after a rice crop has the ability to meet the demands arises for food and nutrition of the growing population. A number of short duration and moisture stress tolerant crops and their improved varieties can be grown in these fallow lands as the climatic and soil condition of the fallows varied from vary small duration to as long as 3 months. Pulses, because of shorter duration and their ability to grow under residual moisture are good candidates for efficient utilization of fallows. However, knowledge and technical gaps in management practices of pulses under fallows and different soil conditions creates hindrance in its proper utilization. Adoption of early and mid early rice varieties in wet season will have a greater window for raising a good crop in dry season particularly the pulses and oilseeds having tolerance to low temperature.

However, energy requirement of different cropping systems varied a lot, different crops and varieties may be suitable for specific locations while going for intensification of the systems. Cultivation of no- till vegetable pea after rice harvest in rice- fallow areas recorded higher green pod yield (5.89 t ha<sup>-1</sup>) resulting in 44 % less energy requirements compared to conventional tillage (Singh et al. 2015). In another study, Das et al. (2008) observed significantly higher net return in rice–french bean system as compared to rice mono-cropping. Farmer's net income enhanced by Rs.29,000 ha<sup>-1</sup> and Rs 21,500 ha<sup>-1</sup> over rice-fallow system due to inclusion of pea (vegetable) and lentil, respectively, in the system following no tillage practice. Singh et al. (2014) suggested that the suitability and profitability of cropping system varies not only with soil type but also with soil series. For example rice–potato produced higher yield in *Lahangaon* series whereas rice–pea in *Teok* series of Upper Brahmaputra valley of Assam.

In rice fallows of eastern India, various pulses, viz. lathyrus, lentil, field pea, blackgram, greengram, chick pea, fababean and oilseeds crops viz. linseed, mustard, and niger etc. can be grown as *utera* crop. Selection of crop and varieties are important



#### Existing system Region Recommended system Pakistan Rice-wheat rice-wheat-greengram-lentil Nepal Rice-wheat Rice-lentil-maize, Rice-lentil-greengram Bangladesh Rice-rice Rice-vegetable pea-rice Non-saline areas of Satkhira Rice-rice Aman rice-mustard-jute district in Bangladesh Aman rice-potato-jute High Ganges River Flood plain Rice-rice Wheat-greengram-rice of Bangladesh Rice-rice Rice-mustard-rice Bangladesh Sandy clay loam soil of Rice-wheat Rice-potato-blackgram, rice-maize Varanasi (UP) (cob) + veg. pea (1:2) - green gramcropping Karnal, Haryana Rice-wheat Rice - vegetable pea - wheatgreengram Punjab Rice-wheat Maize-potato-onion Clay loam soils of Rice-wheat Hybrid rice-gobhi sarson-okra Jabalpur (MP) Rice-chickpea South Gujrat Rice-wheat Rice-sorghum-sorghum ratoon Rice-sweet corn-blackgram Rice-greengram-groundnut Rice-rice High rainfall zone of Rice-fenugreek-okra south Gujarat Rice-rice Krishna –Godavari delta Rice-maize, Rice – mustard areas of Andra Pradesh, Rice-fallow North Coastal Zone of Sunnhemp-rice-blackgram Andhra Pradesh Low land rice ecosystem Rice-maize Rice-soybeanRice-marigold of Karnataka Rice-wheat Jharkhand (irrigated) Rice-potato + wheat (1:1)-Rice-fallow greengram, Rice-potato-greengram Irrigated medium land acid Rice-rice Winter rice-cabbage- greengram soil situation of Assam Rice-chilli-blackgram Rice-rice Irrigated ecosystem in Bihar Rice-tomato-bottle gourd, Ricepotato-onion, Rice-coriander-lady's fingers, Rice-carrot-cowpea Rice-rice Chhattisgarh plains Rice-potato-cowpea Rice fallow Upper Brahmaputra Rice-potatoRice-pea valley zone of Assam Coastal plain zone of Odisha Rice-fallow Rice-ratoon-greengram, Rice-greengram/ blackgram Rice fallow, Khurda Rice-fallow Rice-utera blackgram District of Odisha

#### Table 1. Recommended intensified cropping systems for different regions.



criteria for getting the desire result from *utrea* crop. The results of on-farm trials proved that the blackgram crop was the most feasible crop in coastal Odisha. Crops like sunflower, groundnut, watermelon, lady's finger can be raised with limited irrigation. Medicinal crops like brahmi, bhrungaraj, bhukadamba, panmadhuri, juani, jalabrahmi were found promising in rice based cropping sequence for increasing the profitability of rice farmers. Sunflower found promising in coastal Odisha after rice with limited irrigation.

# 3. MANAGEMENT OF RICE-BASED CROPPING SYSTEM

System productivity of different cropping sequences and systems depends on optimum management of resources by considering system as one unit. Efficient crop establishment methods followed by optimum management of water and nutrients coupled with need based control of various biotic stresses particularly weeds are prerequisites for sustainability of any production system.

#### 3.1. Crop establishment

Crop stand establishment is the most critical operations for ensuring a good crop particularly under rainfed situations. In rice-based cropping system; rice is commonly established by transplanting. Huge amount of water and labour requirements for transplanting reduces profit margins and become less remunerative. Puddling of land makes the land unsuitable for subsequent crop production in transplanted rice. Thus, the rice–rice and rice-wheat systems practised currently are unsustainable. Therefore, management practices with reduced cost of production and increased productivity and lower environmental ill effects are needed which can be achieved switching to conservation agriculture (CA) practices. In heavy clay soils, where there is less chance of land preparation, 'zero tillage crop establishment' following relay cropping or dibble seeding in rows with crops like greengram, blackgram, lathyrus, linseed and lentil are suggested for higher system productivity. Mulching with crop residues like rice straw helps in moisture conservation and early establishment of crops.

From cropping system study in rice-wheat belt, drum seeding (wet bed, unpuddled) resulted in higher grain yield of hybrid rice, whereas direct seeding (dry bed, unpuddled) adopted in the preceding rice crop produced greater yield of wheat, chickpea and Indian mustard. Direct seeding (dry bed, unpuddled) also increased the net returns and benefit:cost ratio. Shweta and Malik (2017) recorded maximum mean grain (4.24 t ha<sup>-1</sup>) and straw (6.24 t ha<sup>-1</sup>) yields of wheat in rice-wheat system when rice was established through direct seeding and nutrient uptake (NPK) by the wheat crop was highest under direct seeded rice due to rice establishment methods.

#### 3.2. Energy management

Energy is the most critical inputs in agriculture and availability of the appropriate energy and its efficient use are pre requisites for sustainable agricultural production. Energy efficiency can be achieved through adaption of suitable cropping system with integrated crop management practices. Energy use efficiency varies with cropping



system, e.g system energy use efficiency was higher in rice-mustard followed by rice-sunflower and rice-maize, while it was lowest in rice-black gram and rice-ragi systems but rice-maize recorded the highest energy productivity followed by ricerice and rice-mustard and the energy productivity was lowest in rice-ragi and ricegingelly (Rao et al. 2014). Energy-use efficiency (EUE), in terms of total output energy/ total input energy, was significantly higher in rice-lathyrus cropping system followed by rice-sunflower + greengram compared to other crop sequences as legumes require much less energy than other crops (Walia et al. 2014). However, the suitability and adaption of cropping system is more dependent on the total energy requirement than the energy use efficiency particularly based on land holding size. For medium and large farmers, jute-potato-rice, rice-potato-rice and rice-potato-sesame are suitable due to higher system productivity but at the cost of higher consumption resulting lower energy productivity. However, jute-wheat and jute-rapeseed-rice are considered to be suitable for small and marginal farmers because of their moderate cost of cultivation and net return with better energy use efficiency (Biswas 2017). Ricesunflower and rice-horsegram cropping systems were energy efficient systems with high net energy despite of having low system productivity (Rautaray et al. 2017).

On the basis of productivity, profitability and energy use efficiency, ricepotato-lady's finger was found to be the best crop sequences for Indo-Gangetic Plains of West Bengal. In red sandy loam soils of northern Telangana, rice-maize and rice-sunflower cropping systems are sustainable cropping sequences as higher production efficiency, input use efficiency and profit was achieved with lower energy input than the other cropping sequences particularly rice-rice.

The energy and monetary budgeting of cropping systems should be done for sound planning of sustainable systems. Rice-wheat system gained 27.7, 21.2, 17.2 and 10.8 per cent higher net return energy than soybean-wheat and pigeon pea-wheat systems, rice-vegetable pea-wheat-green gram and maize-vegetable pea wheat systems, respectively. Adaption of greengram and Sesbania as green manuring crop in rice-wheat cropping systems increases the energy output of the system by 10 and 14% compared to rice-wheat summer ploughing system.

#### 3.3. Nutrient management

Productivity and profitability of a cropping system depends mostly on nutrient management practices. It is essential to have clear-cut idea about the nutrient requirements of component crops, their uptake pattern, soil contribution and use efficiency for developing the optimum nutrient management schedule of an intensive rice-based cropping system. Long term fertilizer experiments indicated decreasing trend of productivity in intensive rice based cropping systems with recommended doses of fertilizers. Therefore, an alternative integrated nutrient management system is required to maintain or improve soil fertility to achieve the desired levels of crop productivity and improved physio-chemical and biological properties and SOC sequestration through optimisation and integration of all available sources of plant nutrients. The INM approach is more suitable in rice based cropping systems compared



to other cropping systems as rice is predominantly grown under submerged soil conditions with greater scope for using different nutrient sources.

Combinations of organic and inorganic fertilizer can augment SOC and other nutrient accumulation and improve crop production under rice based cropping systems (Hossain et al. 2016). Cow dung and manures when combined with NP fertilizer increased SOC content by 0.30 Mg ha<sup>-1</sup> yr<sup>-1</sup> in a rice-wheat rotation. The INM strategy have already results in economically viable, agronomically feasible and environmentally sound sustainable crop production systems by improving soil fertility and C sequestration, and reducing N losses and emission of greenhouse gases. High yield in a rice-wheat cropping system can be obtained through integrated nutrient management by including green manure, legume residues or loppings which can save upto 80-120 kg N ha<sup>-1</sup> or by applying them with 80 kg N ha<sup>-1</sup>, 1 t ha<sup>-1</sup> more grain and about 3t ha<sup>-1</sup> more straw can be produced compared to plots receiving no organic manures/residues. In rice + blackgram intercropping application of 15 kg N through FYM and 20 kg N through chemical fertilizer (urea) + 40 kg P<sub>2</sub>O<sub>5</sub> + 40 kg K<sub>2</sub>O yielded 37% higher REY than the recommended fertilizer dose (Mishra et al. 2012).

Under south Gujarat condition, Mansuri et al. (2016) observed that application of 50% recommended dose of nitrogen (RDN) through farmyard manure(FYM) and 50% bio-compost through improved the availability of nitrogen, phosphorus and potassium in soil. Similarly, application of 50% RDN through chemical fertilizers coupled with 50% RDN through green manuring of azolla or through farm yard manure to *kharif* rice followed by supply of recommended dose of chemical fertilizers to summer rice can be adopted for rice–rice cropping system in coastal Odisha to obtain higher and sustainable yield and maintenance of soil health (Mishra et al. 2017).

Higher system productivity of rice-maize cropping system was achieved with application of 50% NPK through fertilizers + 50% N through *Glyricidia* to rice and 75% RDF to succeeding maize crop. However, the uptake of these nutrients was more with 50% N substitution either through FYM or *Glyricidia* to *kharif* rice, whereas higher nutrient uptake by maize was recorded with 100% NPK supplied as inorganic source. Kumar et al. (2017) suggested that replacing summer rice with maize in rice-rice cropping systems is beneficial in limited irrigation when INM is followed with use of locally available organic nutrient sources i.e. paddy straw/green manure crops.

Site Specific Nutrient Management (SSNM) is another potential option for improving productivity, net income and nutrient use efficiency without depletion of soil fertility in rice based systems in Indo Gangetic Plains. Site specific nutrient management could improve the REY of system by 9.5 to 30%, over blanket recommendation, soil testing based recommendation and farmers practice, respectively (Singh et al. 2015). Site specific nutrient management has successfully been demonstrated in India and proved to be of environmental friendly owing to its balanced and need-based nutrient application.

Conservation agriculture involving zero- or minimum-tillage and crop residue management are key technologies for agro ecological intensification of rice based



cropping systems as it reduces the risk arise out of climate change while making the production sustainable. Mulch can increase yield, water use efficiency, and profitability, while decreasing weed pressure. Use of wheat residue in rice does not have any adverse effect on rice yield if incorporated to soil. Further residue retention under conservation tillage practices also enhanced the system yield in rice-wheat cropping system (Yadav et al. 2017).

Application of 100% recommended dose of fertilizer to the preceding rice crop along with the 'P' of blackgram to rice at sowing substantially may improve the seed yield as well as net monetary benefit in rice-blackgram *utera* cropping sequence. Application of blanket fertilizer reduced the yield and was economically unsuitable. Further application of phosphorous to rice crop can enhance the grain and stover yield of blackgram due to the residual effect.

#### 3.4. Water management

Water availability is an important criterion for selecting components crops in a cropping system particularly in dry season due to non availability of natural rainfall. The water use efficiency can be increased by identification of appropriate crop combinations. More so, agro ecological intensification of rice based cropping systems largely depends upon water availability in dry season. In rice-based cropping system crop water management in rice has a greater impact on the succeeding crop due to change in water regime. Puddling is time consuming, capital intensive and requiring more water and causes subsurface compaction, thereby is not conducive to the succeeding crops. New technologies for saving water, such as alternate wetting and drying, saturation soil culture, raisedbeds and aerobic rice found promising in enhancing water productivity of rice cultivation in South Asia. However, in the coarse texture soils of Punjab a good combination of water management practice and puddling intensity can enhance the water productivity and sustain system productivity. Flooding to field capacity throughout the rice crop considerably reduces the number of irrigations and also gives higher water use efficiency. Besides, the depth of submergence at 3-10 cm is sufficient for the optimum yield and control of most weeds. Alam et al. (2017) observed that the wheat-mungbean-dry seed aman rice system had a very large effect on water productivity and achieved the highest value of 12–17 kg ha"<sup>1</sup> mm"<sup>1</sup>. In sequential cropping, supplemental irrigation after rice had a significant effect on grain yield of winter crops in rice based cropping system and with two supplemental irrigation at tassel initiation and grain filling stage of maize, peg initiation and pod development stage of groundnut, 50% flowering and grain filling of sunflower, crown root initiation and jointing stage of wheat, stolonization and tuberization of potato increased water use efficiency and productivity. Singh et al. (2014) reported that higher field water use efficiency and yield of dry crops after rice with two life saving irrigations in groundnut, lentil, rapeseed and potato respectively. The water use efficiency of rice-lentil-greengram system was higher than rice-wheat-greengram and higher rice equivalent yield was obtained in ricewheat-greengram and rice lentil greengram compared to rice-wheat system. Higher water productivity and lower water use can be achieved in rice by soil mulching in



combination with direct seeded aerobic condition. Further improvement in water productivity can be achieved by reducing area under summer rice in Gangetic flood plains of West Bengal by substituting with other food crops like wheat, maize and pulses which is necessary for sustainability of agroecosystem security and economic growth of the farmers. Enhancing water productivity through water saving technologies is not individual farmer's affairs. Mass awareness programme is required to educate all the farmers of a village or region about modern technologies for water saving as well as yield maximization using modern management practices.

#### 3.5. Weed dynamics and management

Weeds are major biotic stress which affects crop production due to crop weed competition. Weeds are dynamic in nature and weed infestation largely depends on type of crop, cropping system and cultural practices. Cropping system intensification in combination with new management practices has a great impact on the weed flora/ dynamics of the region. Cultural practices like crop rotation, crop geometry, moisture regime, mulching, tillage practice influences the dominance of weed flora and its distribution thus determines the weed control methods. Soni et al. (2012) concluded that relative weed density and weed-flora differed from crop to crop from early stage to maturity of crops. Thus, infestation of severe weeds viz., Phalaris minor in wheat, Chichorium intybus and Rumex spp. and Medicago denticulata in berseem could be minimized by intensified and diversified them with other crops with higher production efficiency. Mishra and Singh (2012) revealed that Echinochloa colona L. and Cyperus iria L. population density can increase in rice due to continuous zero-tillage in rice wheat cropping system. Zero-tillage reduced the population of Avena ludoviciana L. and Chenopodium album L. but increases the density of Medicago hispida compared to conventional tillage systems.

Research results from Modipuram indicated a significant impact of stale seedbed in reducing the weed incidence in rice-wheat system as compared to the traditional seedbed preparation.Crop rotation using weed smothering and land covering crop such as cowpea, soyabean and blackgram reduces the weed population a substantial level. Similarly, intercropping systems involving rice and non-rice crops like greengram, blackgram, pigeon pea or groundnut can help in suppressing the weeds in upland. Intensification of rice-wheat system through inclusion of summer greengram though produces comparable grain yield to summer cowpea or Sesbania green manuring but recorded lower grasses and sedges and weed dry matter. Weed management by using herbicides gaining popularity due to easiness and low-cost involvement, but herbicides are highly crop specific and has residual effect on succeeding crop. Therefore, selection of herbicides for weed management in cropping system particularly in intercrops should consider about the selectivity and residual effect on succeeding crops. In direct seeded rice-wheat cropping system, conventional tillage in both crops and pre-emergence application of butachlor at 1.5 kg ha<sup>-1</sup> followed postemergence application of +2,4-D at 0.5 kg ha<sup>-1</sup> in rice and isoproturon at 0.75 kg ha<sup>-1</sup> + 2,4-D at 0.5 kg ha<sup>-1</sup> post-emergence in wheat produced maximum rice equivalent yield and net returns as compared to other tillage practises and weed control method,



respectively and thus ultimately reduce weed density and weed biomass under the conservation tillage. Application of herbicide bispyribac sodium 25 g ha<sup>-1</sup> at 15 days after transplanting (DAT) followed by one hand weeding at 45 DAT can be recommended for better weed control, growth and yield of transplanted rice without significantly affecting the black gram growth and establishment (Parthipan et al. 2013).

In rice-wheat cropping system, the conservation tillage may have positive effect on the suppression of weeds in wheat. By understanding the nature of weed seed bank, various methods of integrated weed control should be formulated. The adoption of recommended agronomic practices, understanding the nature of the weeds and conservation agriculture can help the farmers to obtain the maximum productivity of rice-wheat cropping system.

#### 3.6. Environmental impact

Rice-rice cropping system is being seen as a major culprit due to higher methane emission from anaerobic condition. However improved management practices such as conservation tillage, crop residue retention and FYM incorporation to soil can improve SOC accumulation which is a major agricultural strategy for mitigating greenhouse gas emissions, enhancing food security and sustainability. Diversification of rice-rice cropping system involving other dry crops can reduce the methane emission from the system but there is possibility of increase in N<sub>2</sub>O from the soil. Therefore, net global warming potential of the system may be balanced. However, Bhattacharyya et al. (2014) reported that rice ecosystem acts as a net agricultural sink rather than a polluter. Highest  $CH_4$  flux was recorded from rice-rice rotation plots and during wet season, higher N<sub>2</sub>O flux was observed from rice-potato-sesame rotation. Higher global warming potential (GWP) of rice-rice cropping system was recorded compared to rice based cropping systems involving other dry crops. Therefore, rice-potato-sesame cropping systems was economically profitable and environmentally safe in eastern Indian condition (Datta et al. 2011).

# 4. TECHNOLOGICAL KNOWLEDGE GAP

There is a great spatial variation in cropping intensity among the various ecosystem and region of India. Punjab has the highest cropping intensity of 189%, followed by Himachal Pradesh (188.1%), West Bengal (185.1%), Haryana (181.3%) and Uttar Pradesh (157.7%). The intensity is low in dry, rainfed regions of Rajasthan, Gujarat, Maharashtra and Karnataka (110-125%). As the average cropping intensity is low in rainfed areas, there is an urgent need for intensification to achieve livelihood security of farm families in those areas. At present, all crop diversification and intensification are primarily based on increase in productivity only without considering the negative impact on environment. Under input based production system, the cost of cultivation is high and in increasing trend and very limited effort has been made to reduce the cost of cultivation. Crop and soil management recommendation are based on individual component crop basis without taking into consideration of carry over effect/mutual



interaction with in the component crops in sequence/system both in time and space. Eastern India is mostly rainfed with about 12.5 mha rice fallows but little effect have been made to bring rice fallows under cultivation. Lack of suitable technology and socio economic problem to bring fallow areas under cultivation are major concerns for sustainability of rice ecosystem.

# 5. RESEARCH AND DEVELOPMENT NEEDS

Intensification and diversification of existing dominant cropping system of the region like rice-rice, rice-wheat, rice-maize, rice-groundnut, rice-rapeseed cropping system with inclusion of short duration pulses/oilseeds in sequence or as an intercrop with due consideration of soil health and environment impact in irrigated ecosystem is the need of the hour. Further, intensification of the existing rice-pulse/oilseed and rice fallow cropping system in rainfed ecosystem has to be done through adoption of resource conserving practices like minimum/zero-tillage, residue retention, mulching etc. Reducing the cost of cultivation/production of the rice based cropping system through farm mechanization, tillage, optimal/precise use of inputs, recycling/reuse of resources can be tested and validated in farmer's field. Development of best management practices to increase resource use efficiency for existing and innovative cropping systems are required for ensuring the sustainability. Developing/intensifying rice fallows through crop adjustment, advancing sowing time for optimal utilization of residual moisture and integrated crop management practices like seed priming, seed treatment etc. maybe studied.

# 6. WAY FORWARD

There is a need to create awareness among the farming community for optimal utilization of resources *i.e.* land, labour, water and energy and ensuring livelihood security through adoption of agro-ecology based intensified cropping system. Appropriate policy may be formulated to incentivise the farming community adopting agro-ecologically intensified cropping system, ensuring availability of the inputs to the farmers at right time. Developing irrigation facility with use of micro irrigation system for efficient water use and enhancing the cropping intensity in the eastern region is another important aspect of the cropping system research and development which should be taken care of at appropriate stage.

#### References

- Alam MJ, Humphreys E, Sarkar MAR and Yadav S (2017) Intensification and diversification increase land and water productivity and profitability of rice-based cropping systems on the High Ganges River Floodplain of Bangladesh. Field Crops Research 209:10-26.
- Baishya A, Gogoi B, Hazarika J, Hazarika JP, Bora AS, Das AK, Borah M and Sutradhar P (2016) Maximizing system productivity and profitability through crop intensification and diversification with rice (*Oryza sativa*)-based cropping systems in acid soils of Assam. Indian Journal of Agronomy 61(3):274-280.



- Biswas B (2017) Cropping system analysis for agricultural sustainability-productivity, economy, ecology and energy use efficiency. Journal of Experimental Biology and Agricultural Sciences 5(3):12-19.
- Bhattacharyya P, Neogi S, Roy KS, Dash PK, Nayak AK and Mohapatra T (2014) Tropical low land rice ecosystem is a net carbon sink. Agriculture, Ecosystems and Environment 189:127-35.
- Cassman KG (1999) Ecological Intensification of Cereal Production Systems: Yield Potential, Soil Quality, and Precision Agriculture. Proceedings of National Academy of Sciences USA 96:5952-5959.
- Datta A, Rao KS, Santra SC, Mandal TK and Adhya TK (2011) Greenhouse gas emissions from rice based cropping systems: Economic and technologic challenges and opportunities. Mitigation and Adaptation Strategies for Global Change16: 597–615.
- Desai LJ, Thanki JD, Gudadhe NN and Pankhaniya RM (2016) Sustainable Productivity and Profitability of Diversified Rice-based Cropping System under South Gujarat condition. International Journal of Science, Environment and Technology 5(3):1100 – 1107.
- Dwibedi SK, De GC, Dhua SR and Dwibedi L (2016) Energetics of rice-ratoon-greengram production system in coastal Odisha. International Journal of Agriculture Sciences 8(9):1096-1099.
- Ghimire R, Lamichhane S, Acharya BS, Bista P, Sainju UM (2016) Tillage, crop residue, and nutrient management effects on soil organic carbon sequestration in rice-based cropping systems: A review. Journal of Integrative Agriculture 15:60345-60347.
- Hassan L, Quddus MA, Khan MAH and Ali M (2015) Intensification of rice based cropping system incorporating short duration oilseed mustard varieties. Journal of Bangladesh Agricultural University 13(1):1-6.
- Hossain M S, Hossain A, Sarkar M A R, Jahiruddin M, Teixeirada Silva JA, Hossain MI (2016) Productivity and soil fertility of the rice-wheat system in the high Ganges river floodplain of Bangladesh is influenced by the inclusion of legumes and manure. Agriculture, Ecosystems and Environment 218:40–52.
- Kumar A, Srivastava LK, Mishra VN, Banwasi R (2017) Major and micro nutrient status of ricechickpea grown in soils of Chhattisgarh plain region of India. Indian Journal of Agricultural Research 51(1):1-8.
- Lal B, Gautam P, Panda BB, Raja R, Singh T, Tripathy R, Shahid M and Nayak AK (2017) Crop and varietal diversification of rainfed rice based cropping systems for higher productivity and profitability in Eastern India. PLoS ONE 12(4): e0175709.
- Laurance WF, Sayer J and Cassman KG (2014) Agricultural expansion and its impacts on tropical nature. Trends in Ecological Evolution 29: 107-116.
- Malik AI, Nasim M, Flower K and Hossain MA (2017) Cropping system intensification: vegetable pea can replace fallow between rainfed monsoon rice and irrigated spring rice. The Journal of Agricultural Science 155(8):1287-1298.
- Mansuri RN, Thanki JD, Sindhi SJ, Desai LJ and Singh N (2016) Physio-chemical properties of soil as affected by integrated nutrient management in rice-chickpea cropping sequence under south Gujarat condition. Advances in Life Sciences 5(12):27-32.
- Mishra A, Behera B, Pal AK, Mahanty SK, Rath BS, Subudhi CR, Nayak SC and Sahoo N (2012) Performance of rice and blackgram with different nutrient management practices in rainfed upland. Oryza 49(4):273-279.
- Mishra KN, Patra AK, Garnayak LM, Mohanty AK, Swain SK (2017) Long-term effects of integrated nutrient management on productivity and soil properties of rice (*Oryza sativa*)– rice cropping system in coastal Odisha. Indian Journal of Agronomy 62(3):239-246.



- Naresh RK, Rathore RS, Dwivedi A, Kumar V, Kumar A, Kumar V, Kumar M, Kumar A, Tyagi S, Singh O and Gupta RK (2017) Bed planting system promotes crop diversification for improving livelihoods in western Uttar Pradesh, India. International Journal of Current Microbiology and Applied Sciences 6(2):1580-1590.
- Parthipan T, Ravi V, Subramanium E and Ramesh T (2013) Integrated weed management on growth and yield of transplanted rice and its residual effect on succeeding Black gram. Journal of Agronomy 12(2):99-103.
- Pokhrel A and Soni P (2017) Performance analysis of different rice-based cropping systems in tropical region of Nepal. Journal of Environmental management 197:70-79.
- Prasad D (2016) Diversification of existing rice-based cropping system for sustainable productivity under assured irrigation condition for Chhattisgarh plains. International Journal of Multidisciplinary Education and Research 1(3):85-88.
- Prasad D, Yadava, MS and Singh CS (2013) Diversification of rice (*Oryza sativa*)-based cropping systems for higher productivity, profitability and resource-use efficiency under irrigated ecosystem of Jharkhand. Indian Journal of Agronomy 58(2):77-83
- Rao UA, Sridhar TV, Adi Lakshmi D and Raju SK (2014) Identification of viable rice based cropping systems for double cropped delta areas of Andhra Pradesh. International Journal of Science and Nature 5(3):512-514.
- Rautaray SK, Mishra A and Verma OP (2017) Energy efficiency, productivity and profitability of rice (*Oryza sativa* L.) based cropping systems for selected conservation practices. Archives of Agronomy and Soil Science 63(14):1993-2006.
- Roy DC (2016). Trend in changes of crop sequences due to water problems ensuring food security in Gangetic flood plain in eastern India. International Journal of Bio-Resource, Environment and Agricultural Sciences 2(1):251-260.
- Samant TK (2015) Major crops, cropping systems and farming systems in mid central table land zone of Odisha. International Journal for Research in Agricultural and Food Science 2(1):90-98.
- Shweta and Malik M (2017) Improving wheat productivity in rice-wheat cropping system through crop establishment methods. International Journal of Pure and Applied Bioscience 5(3):575-578.
- Sharma S, Rajendran R, Ravi V, Panneerselvan P and Janarthanan P (2016) Improving resource use efficiency of cereal based cropping systems with integration of best management with conservation agriculture under changing agricultural scenarios in Cauvery Delta of Tamil Nadu. Journal of Ecosystem and Ecography 6:213 - 25.
- Singh D, Bhaskar BP, Baruah U and Sarkar D (2014) Evaluation of rice (*Oryza sativa L*.) based cropping systems in major soil series of upper Brahmaputra Valley Asom. Global Journal of Science Frontier Research: D Agriculture and Veterinary 14(6):on line.
- Singh RD, Shivani, Khan AR and Chandra N (2010) Sustainable productivity and profitability of diversified rice-based cropping systems in an irrigated ecosystem. Archives of Agronomy and Soil Science 58(8):859-869.
- Singh VK, Shukla AK, Dwivedi BS, Singh MP, Majumdar K, Kumar V, Mishra RP, Rani M and Singh SK (2015) Site-specific nutrient management under rice-based cropping systems in Indo-Gangetic Plains: Yield, profit and apparent nutrient balance. Agricultural Research 4(4):365-377.
- Soni M, Upadhyay VB and Singh P (2012) Weed dynamics and production efficiency of rice-based cropping system. Indian Journal of Weed Science 44(1):21–25, 2012.



- Srilatha M, Sharma S and Kumar H (2015) Influence of long term use of fertilizers and manures on available nutrient status and inorganic "Phosphorous" fractions in soil under continuous rice-rice cropping system. International Journal of Advanced Research 3(6):960-964.
- Yadav G S, Datta R, Pathan SI, Lal R, Meena RS, Babu S, Das A and Bhowmik SN (2017) Effects of conservation tillage and nutrient management practices on soil fertility and productivity of rice (*Oryza sativa* L.)-rice system in north eastern region of India. Sustainability 9:1816 32.\*



# Integrated Rice-based Farming Systems for Enhancing Climate Resilience and Profitability in Eastern India

## A Poonam, S Saha, PK Nayak, BS Satapathy, M Shahid, AK Nayak, R Tripathy, NN Jambhulkar, GAK Kumar, B Mondal, PK Sahu, SC Giri, M Nedunchezhian, U Kumar and SK Lenka

# SUMMARY

Majority of the rice farmers in eastern India possess small land holding (<1ha), which is the only primary source of farm family income. Despite exploiting their land extensively they often fail to achieve the target appreciably owing to high risk involved in the natural calamity. Rice ecosystem in the eastern region is diverse and rich in natural resources but the extremely fragileness of the ecosystem and small and marginal land holdings call for diversification of the persisting/ conventional rice farming for efficient resource management. Adoption of the most compatible multi-crop enterprises into a small system holding would greatly improve the production system in the risk prone ecology and will ensure sustainable food, economic and employment in the small farm thus restricting the migration of farming community to the metro cities for construction and other non-agriculture work.

# 1. INTRODUCTION

Farming family in tropical India is mainly dependent on rainfed farming with high risk of weather uncertainty. In a constant struggle to survive, the small and marginal farmers over the years have evolved techniques which have benefited them immensely. But without knowing the scientific basis of such integration they have been practicing the system for a long time. In India, traditionally, farming has been family based and majority of them are smallholders. The success of farming family lies not in 'specialization' but in practicing farming to meet diverse household needs rather than market opportunities alone. Hence, income from seasonal field crops alone in small and marginal farms is hardly sufficient to sustain the farming family.

Rice being the staple food is grown in the country in around 43.5 million ha (Mha) under various ecologies of which about 50% area is rainfed. More than 80% of the rice farmers belong to small and marginal groups and the average per capita land holding in India is only about 0.17 ha. In view of the population growth, competition of land with industrialization and urbanization, declining farm holding size and the dietary nutrition requirement of the farm families, it is necessary to look for the optimum use of resources through shift from conventional rice farming to integrated farming systems. Rice based farming system involving rice, other field and horticultural crops, agro-forestry, fish birds livestock and further income generating enterprises will be



the right approach in this respect and will be more relevant in the risk prone rainfed ecologies which are mostly located in the eastern part of the country. Because of reducing per capita availability of land in India, there is no further scope for horizontal expansion of land for increased food production. Hence, the best possible allocation of resources and their intelligent management is important to reduce the risk related to land sustainability. Integrated farming system is the potential approach and powerful tool for management of vast natural and human resources in developing countries including India to meet the various objectives of, competitiveness, food security, poverty reduction and sustainability of small and marginal farmers.

Rice farmers in eastern part of India generally work in various risk prone environments leading to low rice productivity. Available resources for modest increment is due to lack of appropriate understanding of interaction and linkages between the components under traditional rice farming system; manpower is underutilized for employment and hence, rice growers are economically poor. Integrated farming system (IFS) has been advocated as one of the tool for harmonious use of inputs and their compounded response to make the agriculture in the region profitable and sustainable.

The Indian economy is predominantly based on agriculture, which is facing a serious challenge in terms of the sustainability and profitability of farming due to the declining trend in size of land holding. The average size of the landholding has declined to 1.1 ha during 2010-11 from 2.28 ha in 1970-71. If this trend continues, the average size of holding in India would be mere 0.68 ha in 2020, and would be further reduced to 0.32 ha in 2030 (Agriculture Census 2010). As per estimates, more than 95% of the holdings will be under the category of small and marginal holders in 2050. Hence, it is necessary to develop strategies and agricultural technologies that enable adequate employment and income generation, especially for small and marginal farmers who constitute more than eighty per cent of the farming community.

Under the gradual shrinking of land holding, it is needed to integrate land based enterprises like minor livestocks, field and horticultural crops, etc. within the biophysical and socio-economic environment of the farmers to make farming more profitable (Behera et al. 2004). In addition, the dependence upon a few crops in combination with a high biotic and abiotic risk of crop failure exposes the farmers to a high degree of variability with respect to yield and income and therefore risks (Ashby 2001). Further, few authors indicated that commercial farming systems are a threat to the environment through a loss of genetic diversity and the possible negative impacts of these systems and their associated inputs.

It becomes difficult for the small and marginal farmers to sustain with the single farm enterprise unless resorting to integrated farming systems (IFS) for the generation of adequate income and year round employment within their small farms (Mahapatra 1992). Decreasing land-man ratio, poor socio-economic condition of farmers, vagaries of monsoon, new risks from environmental deterioration, population pressure and rapidly changing agricultural input and output markets through globalization, high degree of vulnerability in the rural area proved to be not sustained farmer's livelihood



mainly when they are solely dependent on traditional agriculture on their small piece of lands. Therefore, agriculture diversification is utmost important for improving their livelihood and reducing vulnerability.

#### 1.1. Integrated farming system-a promising approach

Integrated farming system is the potential approach and powerful tool for management of vast natural and human resources in developing countries, including India to meet the multiple objectives of poverty reduction, food security, competitiveness and sustainability of small and marginal farmers' livelihood. The approach aims at increasing income and employment from small-holding integrating various farm enterprises and recycling crop residues and by-products within the farm itself (Behera and Mahapatra 1999; Singh etal. 2006). Under the gradual shrinking of land holding, it is necessary to integrate land-based enterprises such as dairy, fishery, poultry, duckery, apiary, field crops, vegetable crops and fruit crops within the bio-physical and socio-economic environment of the farmers to make farming more profitable and dependable (Beheraetal.2004).Integrated farming systems are often less risky, because if managed efficiently, they benefit from synergisms among the enterprises, leading to diversity in produce and environmental soundness (Pullin 1998).

The objective of this Chapter is to analyze the traditionally practiced farming systems nationally /internationally and how the progress has been made in terms of different improved models/cropping systems for sustainable, food, nutrition, employment generation and income of the small and marginal rice growers in diverse agro-ecological situations.

# 2. STATUS OF RESEARCH/KNOWLEDGE

The cultivation of almost 90% of the world's rice crops in irrigated, rainfed and deep-water systems equivalent to about 134 million hectares offers a suitable environment for fish and other aquatic organisms. The different integrations of rice and fish farming–either on the same plot, on adjacent plots where by-products of one system are used as inputs on the other, or consecutively – are all variations of production systems that aim to increase the productivity of water, land and associated resources while contributing to increased fish production. The integration can be more or less complete depending on the general layout of the irrigated rice plots and fishponds.

Asia accounted for about 90% of 672 Mt of rice produced in the world as of 2010 (FAO 2012). Rice farming in Asia used to be characterized by small scale, labourintensiveness and on-site recycling of green and animal manures. Although rice farming is still labour-intensive in remote areas of Asian developing countries, it has rapidly been mechanized and agrochemicals- intensive in the name of agricultural modernization and green revolution. In fact, the so-called green revolution has largely resulted from industrial monoculture, genetically modified crops and the excess use of agrochemicals, which caused agricultural land degradation globally. Furuno



(2001)formulated the idea of systematically integrating rice with ducks, based on the notion that two products highly complementary to each other can be jointly produced. In the integrated rice–duck farming (IRDF) system, rice paddies provide food (weeds and pests) for ducks, and ducks play a role in fertilizing rice plants. Integrated farming systems are effectively systems that have traditionally been undertaken by farmers in countries that include Indonesia, China, Malaysia, Vietnam, Rwanda and Thailand (Praphan 2001). However, in many countries these traditional systems have been replaced by the establishment of commercial cash and staple crop production systems that have been promoted by governments (Ruaysoongnern and Suphanchaimart 2001).

As regards the general scale of rice–fish culture, China is the main producer with an area of about 1.3 Mha of rice fields with different forms of fish culture, which produced 1.2 Mt of fish and other aquatic animals in 2010. Other countries reporting their rice–fish production to FAO include Indonesia (92000 t in 2010), Egypt (29 000 t in 2010), Thailand (21000 t in 2008), the Philippines (150 t in 2010) and Nepal (45 t in 2010). Trends observed in China show that fish production from rice fields has increased thirteen fold in the last two decades, and rice–fish culture is now one of the most important aquaculture systems in China, making a significant contribution to rural livelihoods and food security.

Rice-fish farming is being tried and practiced in other countries and continents although to a lesser extent. Apart from Asia, activities have been reported from, among others, Brazil, Egypt, Guyana, Haiti, Hungary, Iran (Islamic Republic of), Italy, Madagascar, Malawi, Nigeria, Panama, Peru, Senegal, Suriname, the United States of America, Zambia, and several countries in the Central Asia and Caucasus region.Ricefish systems are practiced in China, Egypt, India, Indonesia, Thailand, Vietnam, Philippines, Bangladesh and Malaysia. The rice-fish systems are important in these areas because they provide food security, reduce the impact of agriculture on the environment, and may be less affected than conventional systems by climate change. Integrated rice-fish production can optimize resource utilization through the complementary use of land and water. This practice also improves diversification, intensification, productivity, profitability, and sustainability of the rice agro-ecosystem.

In eastern India about 70% of farmer community comes under the marginal and small farmer category (GOI 2009). Farmers under these categories are economically poor and work in diverse risk prone environments. The income from rainfed rice crop and other seasonal field crops on small and marginal farms is hardly sufficient to sustain their family. Integrated farming system (IFS) has been advocated as one of the tool for harmonious use of inputs and their compounded response to make the agriculture in the region profitable and sustainable. Integrated farming systems aim at an appropriate combination of farm enterprises like field crops, dairy, piggery, poultry, apiculture, goatery, mushroom cultivation etc. for a productive, profitable and sustainable agriculture. IFS interact appropriately with the environment without dislocating the ecological and socio-economic balance on one hand and attempt to meet the farmers need on the other. Thus, IFS is a reliable way of obtaining high



productivity with substantial nutrient economy in combination with maximum compatibility and replenishment of organic matter by way of effective recycling of organic residues/ wastes etc. obtained through integration of various land based enterprises (Gill et al. 2010; Sanjeev et al. 2011) An experiment on paddy cum fish culture was started in west Bengal in 1945 in an area of 691.16 acres adjoining the paddy fields and it was remarkably noted that the growth of tank fishes was slower than those liberated in the paddy fields. The then rice committee of FAO in 1948 strongly advocated the practice of fish culture in the rice field for increased production of rice. The farmers of the north-eastern part of India, which includes seven states viz. Assam, Arunachal Pradesh, Nagaland, Meghalaya, Mizoram, Manipur and Tripura cultivate rice as their staple food and a fish crop is traditionally raised only from the paddies of rainfed lowlands (both shallow and deepwater). Traditional rice-fish production systems have an important socioeconomic part in the life of the farmers and fishers in the region. The practice of integrated farming system recorded higher mean average net return (Rs. 3,06,875), gross return (Rs. 3,88,375) and benefit cost ratio (4.58) over farmers practice.employment generation in the farming system under Integrated farming system was 193 days in a year from the Bellari district of Karnataka (Yogeesh et al.2016).Integration of various agricultural enterprises viz., cropping, animal husbandry, fishery, forestry etc. not only supplement the income of the farmers but also help inincreasing the family labor employment through out the year (Javanthi2002).

# 2.1. Improved integrated rice -fish farming system

Eastern India, in particular with about 5.6 m ha irrigated area and 14.6m ha rainfed lowlands of the total 26.58 m ha rice area, offers high potential for rice-fish farming system, especially in view of the resources, food habits and socio-economic needs of the people. Rice-fish farming system with higher water and land productivity and employment opportunities can ensure food, nutrition and livelihood security for the farming communities, particularly for the largest groups of small and marginal farmers. Rice-fish culture systems can be mixed or concurrent, sequential or rotational. However, the techniques differ based on the physical, biological and socio-economic profiles of the target agro-ecosystem.

**2.1.1. Model I: Rice–fish–livestock-horticulture based farming system for rainfed lowland areas:** In order to improve and stabilize farm productivity and income from rainfed water logged lowland areas, national Rice Research Institute, Cuttack has developed an adoptable technology of rice-fish diversified farming system. Farm size may vary from minimum of about one acre to one hectare or more. Field design includes wide bunds (Dykes) all around, a pond refuge connected with trenches on two sides(water harvesting come fish refuge system) and guarded outlet. The approximate area allotments will be, 20 % for bunds, 13 % for pond refuge and trenches and rest 67 % for main field. The pond refuge measures 10 m wideand 1.75m deep constructed in the lower end of the field. The two side trenches of 3 m width and average 1 m depth have gentle(0.5%) bed slope towards the towards the pond refuge.





Fig. 1.Rice –fish–livestock-horticulture based farming system for rainfed lowland areas

Small low cost (Thatched/asbestos top)duck house and poultry unit are constructed on bunds with a floor space of about 1.5 sq.ft. for each duck and 1 sq.ft. for each poultry bird. Poultry unit may be projected upto 50% over the water in the pond refuge to utilize the dropping as fish food and manure in the system. In such case birds can be housed in cages made of wire net. A small goat house is made on the bund with floor space of about 2 sq.ft for each animal (Fig. 1 & 2).

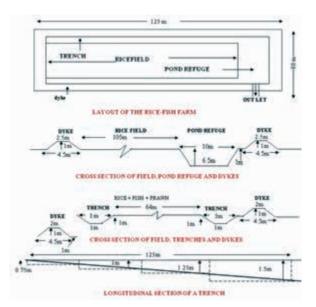


Fig.2. Layout and transverse section of rice based integrated farming system for rainfed lowlands.

#### i. Production Technology

Production Technology broadly involves growing of improved photo-period sensitive semi tall and tall wet season rice varieties with field tolerance to major insect pest and diseases. The suitable rice varieties are Gayatri, Sarala, CR Dhan 500, CR Dhan 505, Jalmani, Varshadhan for Odisha, Sabita, Jogen, Hanseswari for West Bengal, Sudha for Bihar, Madhukar and Jalpriya for eastern Uttar Pradesh and Ranjit, Durga and Sabita for Assam. Management of insect pest in rice crop is done with the use of sex pheromen traps, light traps and botanicals (Netherin/Nimbicidin spray at 1%). Indian major carps (Catla, Rohu, Mrigal) *Puntiussarana*, exotic carps (common carp, silver carp silver barb) and fresh water giant prawn (*Macrobrachiumrosenbergii*) fingerlings of 3-4" size and prawn juveniles of 2-3" size are released in a ratio of 75 %

280



and 25 %, respectively at 10,000 per hectare of water area after sufficient water accumulation in the refuge and in the field. Fish and prawn are regularly fed at 2% of total biomass with mixture containing 95% of oil cake +rice bran (1:1) and 5% of fish meal. After rice, various crops like watermelon, mung sunflower, groundnut, sesame and vegetables are grown in the field with limited irrigations from the harvested rainwater. On bunds different seasonal vegetables are cultivated round the year including creepers on the raised platform, spices and pineapples are grown in shades. The fruit crops on bunds include varieties of dwarf papaya, banana T x D coconut and arecanut. Flowers like tuberose marigold etc. are also cultivated on the bunds. Both straw and ovster mushroom cultivation are done in the thatched or polythene enclose. Bee rearing is practice in 2-3 bee boxes on bunds. Agro-forestry component on the bund include short term plantation of mainly Accacia spp. (A. mangium, A. auriculiformes). Animal component constitutes improved breeds of duck, poultry birds and goats. Ducks are raised in the rice field upto the beginning of flowering stage and later in an enclose in pond refuge till the harvest of rice crop. Live Azolla is released @ 0.5 - 1.0 t ha<sup>-1</sup> and is maintained to supplement duck feed and also to some extent fish feed, besides nutrition to the rice crop. Fresh water pearl culture is integrated in the system using the host mussel (*Lamellidensmarginalis*) which is normally available in the lowland rice ecology. Components can however, be included in the system based on location –specific requirements are grown along with the rice crop and later in the refuge after the rice crop is harvested.

#### ii. Productivity and economics

The rice fish farming system can annually produce around 16 to 18 t of food crops, 0.6t of fish and prawn, 0.55 t of meat, 8000-12,000 eggs besides flowers, fuel wood and animal feed as rice straw and other crop residues from one hectare of farm. The net income in the system is about Rs. 76,000 in the first year. Subsequently, this increases to around Rs. 1,30,000 in the sixth year. This system thus increases farm productivity by about fifteen times and net income by 20 folds over the traditional rice farming in rainfed lowlands (Table 1). The rice fish system also generates additional farm employment of around 250–300 man-days ha<sup>-1</sup>year<sup>-1</sup>.

S. No	Particular	Amount (Rs.)
1.	Construction of pond refuge and trenches and dykes (2000 cm x 35)	70,000
2.	Constriction of platforms 16 No. @ 200/-	3200
3.	Pit digging, planting of fruit and silvicultural plants (125-130 No.)	4000
4.	Cost of seeds/seedlings/saplings	8,000
5.	Cost of FYM/vermicompost	5000
6.	Cost of fingerlings	8000
7.	Cost of fish feed	5000
8.	Small farm implements/equipments	5000
9.	Labour 400 man days @ Rs. 150	60,000
	Total	1,68,200

#### Table 1. Cost of raising rice-fish-horticultural model in 1.0 ha area at NRRI.



**2.1.2. Model II: Rice-fish-prawn-horticulture-agro-forestry based farming system for deep water:** With the aim of enhancing farm productivity in deep water areas (5-100 cm water depth), a multi-tier rice-fish-prawn horticulture crops-agro-forestry based farming system model has also been developed in 0.06 hectares area at NRRI, Cuttack.

#### i. Production Methodology

The design of the system includes land shaping in the form of uplands(tier I and tier II) covering about 15% of field area followed by rice field area of 40% as rainfed lowland (tier III) and deep water (tier IV). This rice field is connected to a micro water shed cum fish refuge (pond) of 20% area for growing of fish and prawn with the rice crop. Raised and wide bunds are made all around using 25% of the farm area. The production technology includes growing of high yielding varieties of rainfed lowland rice (Gayatri, Sarala)in teir III and deep water rice (Durga and Varshadhan) in tier IV along with the fish and prawn during wet season. Dry season crops like sweet potato, mung, sunflower, groundnut, vegetables are grown after lowland rice in tier III. Dry season rice is cultivated after the deep water rice is harvested in their IV. Harvested rain water in the pond refuge is used for irrigation of the dry season crops. Improved varieties of perennial (mango, guava, sapota) and seasonal fruit crops (Papaya, Banana, Pineapple) are grown in upland (tier I). Round the year different seasonal vegetables and tuber crops (sweet potato, elephant foot yam, yam bean, colocasia and greater yam) are cultivated in tier II(Upland). Agro-forestry (Acaciamangium) and plantation crops (coconut and areca nut) are planted on the northern side of the bunds. Greater yam is grown with the support of trunk of agro forestry tree. The productivity of the system is about 8 t of rice crop per hectare, one tone of fish and prawn per hectare, 20-25 t of vegetablesha<sup>-1</sup> and 8.5 to 51.7 t of tuber cropsha<sup>-1</sup>. The cropping intensity in this system greatly increases to 170% in field and 360% in the upland.

#### ii. Productivity and economics

Multi-tier rice fish horticulture based farming system can annually produce about 14-15 t of food crops, 1 t of fish and prawn, 0.5 -0.8 t of meat, 10000-12000 eggs in addition to flowers and 3-5 t of animal feed from 1 hectare farm area. The productivity of food crops further increases to 16-17 t besides, 10-12 t of fiber/fuel wood from eight year onwards due to addition of produce from perennial fruit crops and agro-forestry components. The net income in this system is around Rs. 1,00,000ha<sup>-1</sup> in the first year. This will increase to Rs. 1,50,000 or more from the eight year onwards.

**2.1.3. Model III: Rice-based integrated farming system under irrigated condition:** With the objective of improvement of livelihood of small and marginal farmers, rice based integrated farming system model for irrigated areas has been developed at NRRI, Cuttack.

#### i. Production Methodology

About an acre of integrated farm area has been reoriented for the farming system of which 30% of the area is converted to two rice plus fish fields of 600 sq.m area each with a refuge of 15% area and another 30% area is developed into two nursery fish

282



ponds of equal size of fingerlings rearing (Fig. 3). The remaining 40% (1500 m<sup>2</sup>) area is utilized as bunds for growing vegetables, horticultural crops and agro-forestry. Three rice crops are grown in the sequence of kharif rice (Sarla/Durga) followed by rabi rice

(Naveen/Shatabdi) and then summer rice(Vandana/ Sidhant). Yellow stem borer pest is controlled by using sex pheromone traps or by applying 1% Nethrin/ Nimbecidine. Fish culture is taken up with catla, rohu and



Fig. 3. Transverse section of the rice based integrated farming system for irrigated area

mrigal species. The fish fingerlings are reared in the two nursery ponds and are used for culture with rice crop in the system. The excess fingerlings are sold out. On the bunds agro-forestry plants like teak, Accacia, sisoo, neem, aonla and bamboo are planted on the northern and southernbunds. Horticultural crops such as banana, papaya and arecanut are grown on the bunds. Pineapple and spices are cultivated in the shade. Flowers like marigold,hibiscus and jasmine are also cultivated in the western bund in 50m<sup>2</sup> area. Two plants of lemon and each of guava, jackfruit, mango and litchi are also planted on the southern bund near the farm house to meet the household requirement. One poultry and one duckery unit are integrated in the system in which 40 poultry birds are raised during the dry seasons(October to April) and 20 ducks are reared during the wet season (July to December).

#### ii. Productivity and economics

Three crop of rice yields 800 to 1000 kg of grain per year. Entire produce is sufficient to cater the need of the small farm family. The straw is used for the cattle feed, mushroom base and roof of the farm house. Rest of the straw is sold to earn Rs. 500-1000 per year. After 2-3 months of rearing, fish fry worth of Rs. 4000-5000 is sold to the other farmers. Fish are harvested according to the need after the size becomes 250-300 g after 6 months or 0.5-1.0 kg after a year. The income from fish rearing in the system is Rs. 20,000. Pulses (mungbean, blackgram and pigeonpea) taken on the slope and bunds are just enough to meet the protein requirement of the farm family.

#### 2.2. On Station Research

**2.2.1. Rice-ornamental fish culture:** In order to utilize the rice ecology for value added aquaculture, the technique of breeding and culture of ornamental fishes in irrigated lowland rice field has been developed at NRRI, Cuttack (Fig 4.). The rice field has been renovated to make a pond refuge and raise bunds all around. Ornamental fishes like Blue gourami, Red gourami, Pearl gourami, Guppies are bred and cultured with rice



Fig. 4. Rice + ornamental fish system under irrigated lowlands.



(lowland varieties) crop during wet season. During the dry season, rice (Naveen) cropwas grown along with ornamental fishes with irrigation. About 25,000-6,00,000 ornamental fishes ha<sup>-1</sup>were produced in the system, in addition to 3.5t and 5.0t of rice grain during wet and dry seasons, respectively (Anonymous 1999).

**2.2.2. Lowland weeds and their bio-control:** Weed flora of different rainfed lowland ecosystems were studied with special reference to rice-fish system at the farm of NRRI, Cuttack revealed that with increase of water depth, the weed flora decreased by 39% under rice–fish system indicating fish as a potential bio-control agent for aquatic weeds.

The study conducted by Sinhababu et al. (2013) suggested that exotic carps (grass carp, silver barb and common carp in order) were more effective than Indian carps for control of weed in rainfed lowland rice fields and among the Indian carps, rohu showed potential for weed control. Under the categories of grassy, sedges, broadleaf and aquatic weeds total 13 major weeds were observed in the rice fields. Grass carp reduced maximum weed biomass with weed control efficiency (WCE)of 63% at 60 days after transplanting (DAT) and 62% at 100 DAT followed by silver barb and common carp (Table 2). Among the Indian carps, only rohu was effective in control of weeds (WCE 23% at 60 DAT).

Treatments	Weed biomass (gm <sup>-2</sup> ) 60DAT	WCE (%) 60DAT	Weed biomass (g m <sup>-2</sup> ) 100DAT	WCE (%) 100DAT
Rice + grass carp	0.28 <sup>d</sup>	63.34ª	13.87 <sup>d</sup>	62.31ª
Rice + silver barb	0.32 <sup>cd</sup>	60.54ª	15.62 <sup>cd</sup>	56.55 <sup>ab</sup>
Rice + common carp	0.43°	46.89ª	21.72°	41.81 <sup>b</sup>
Rice + rohu	0.61 <sup>b</sup>	23.10 <sup>b</sup>	28.40 <sup>b</sup>	23.22°
Rice + catla	$0.77^{a}$	4.99°	32.86 <sup>ab</sup>	8.49°
Rice + mrigal	0.76 <sup>a</sup>	3.44°	31.75 <sup>ab</sup>	11.23c
Rice	$0.80^{a}$	-	36.42a	-

Table 2. Weed biomass and weed control efficiency (WCE) in rice alone and rice-fish fields.

Source:Sinhababu et al. (2013)

**2.2.3. Methane and nitrous oxide emission from rice–fish fields and refuge tank:** Datta et al. (2009) observed that  $CH_4$  emission from rice field plots sown with the two rice cultivars, with or without fish, varied considerably (cv = 17%). Methane emission was low in all the plots up to 30 days after sowing (DAS). Presence of fish resulted in an increase in  $CH_4$  emission from both the rice cultivars with two sharp peaks recorded at flowering and maturity stages of the rice crop. The mean  $CH_4$  emission (mg  $CH_4$  m<sup>-2</sup> h<sup>-1</sup>) from sowing till harvest followed the order: Varshadhan + fish (2.52) >Durga + fish (2.48) >Durga (1.47) >Varshadhan (1.17). Cumulative  $CH_4$  emission was highest in the treatment Varshadhan + fish (96.33 kg ha<sup>-1</sup>) while the lowest emission was reported in field plots planted to cv. Varshadhan without fish (45.38 kg ha<sup>-1</sup>). Thus, percentage increase in  $CH_4$  emission as a result of fish rearing was 112 in case of cv. Varshadhan



and 74 in case of cv. Durga. Methane emission from the pond refuge followed a similar pattern as that from rice fields. On the contrary, Unlike  $CH_4$ ,  $N_2O$  emission flux from rice fields exhibited a peak almost immediately after germination and stand establishment, at 30–36 DAS and declined thereafter (Fig.2). As a whole,  $N_2O$  fluxeswere comparatively low during the entire cropping period become greater only towards maturity of the rice crop when the floodwater receded and the field started drying.

**2.2.4.** Physio-chemical parameters under rice fish system: Plots from an average of 28.51-28.94 °C in the different field plots in the morning (at 9:00 h) to around 32.23-34.63 °C in the afternoon (at 15:00 h). Field plots without fish exhibited significantly higher range of water temperature. The mean pH values also exhibited significant differences (p<0.05) between the mean water temperature increased considerably during the course of the day.

**2.2.5. Fish Growth and Performance:** Indian major carps, exotic carps and fresh water prawn was reared at a density of  $0.6m^2$  (1 Prawn: 8 Fish) in the system. Among the fishes, common carp was found to attain maximum size(2.4 kg) followed by Catla (2.0 kg) and the prawn attained asize of 100 to 270g. Sampling and partial harvesting indicated an estimated productivity of fish and prawn as 350 kg per ha per season from the rice-fish system.

2.2.6. Validation and adoption in the farmer's field: Since last two decades, rice-fishhorticulture based farming system was developed in different villages/blocks of Jagatsinghpur and Puri districts of Orissa, under different project funds. Recently during 2014 and 2015 a farmer Sri.KunjoMullick, from Jagasinghpur district belonged to small farmers category developed his rice growing farm into rice fish integrated farming system under the guidance of NRRI experts. His rice field was prone to flood during wet season due to poor drainage, back water and remain waterlogged for nearly 4-5 months. Rise in salinity level was another problem during end march till monsoon. The farmer mostly grew local rice varieties with low inputs and could get very low rice produce (0.8-1.0 t ha<sup>-1</sup>) and hence could not sustain his livelihood. An initial investment of Rs 72,000 was done by the farmer for establishment and shaping of his rice area (Total 4 acres) into watershed/pond area of about 1 acre, where he was advised to put the Indian major carps and the dug out soil was transformed into raised dykes where the farmer took banana, coconut and vegetable crops like cowpea, pumpkin, leafy greens, drum stick etc. Poultry and duckery was taken in the house made with locally available materials. At the end of the year he could get the net income of Rs. 1,50,000 with the cost benefit ratio of 1:2.08.

# 3. KNOWLEDGE GAPS

Traditional agriculture is known to cause environmental degradation because it involves intensive tillage, when practiced in areas of marginal productivity. Technologies and management strategies that can inflate productivity need to be developed. At the same time, ways need to be found to preserve the natural resource



base. Within this framework, an integrated farming system represents a key solution for enhancing overall production and safeguarding the environment through judicious and efficient resource use. Though many farmer, practices integrated farming system traditionally in their homestead land but is not foolproof in combating climatic vagaries and is mostly non interactive. The available resources in these areas, for modest growth in land productivity is not utilized efficiently to reduce the risk related to land sustainability *vis-a* –*vis* employment problem. Poor understanding of interaction and linkages between the components under rice based integrated farming system is the main reason for underutilization of resources and thereby unutilized rural employment leads to poor economic condition of the farmer. The integrated nature of goal-based modelling and the opportunity to play with the system might enhance learning about the different components, their mutual relations and the potentials of the farm system.

# 4. RESEARCH AND DEVELOPMENT NEEDS

Climate change is already happening and its effects, especially on India's rural communities are particularly adverse. Although integrated crop-livestock systems have been practiced globally for millennia, in the past century, farmers have tended toward increased specialized agricultural production for better profitability, concerning about natural resource degradation, stability of farm income, long-term sustainability. Revitalizing integrated crop-livestock systems could foster crop diversity, better managements of selected areas of the landscape to achieve multiple environmental benefits. Integrated systems inherently would utilize animal manure, mutual benefits of crop and livestock's which enhances soil tilth, fertility, and C sequestration, and adoption would enhance both profitability and environmental sustainability of farms and communities. The system components combination complexity and potential for public benefit justify the establishment of a new national or international research initiative to overcome constraints and move toward greater profitability and sustainability. The approach to climate-resilient agriculture will help increase the response capacity of farmers and the resilience of the ecosystem, and reduce their risks to climate change. The need is to highlight the key issues and understand the practical challenges that must be addressed to build the capacities of rural communities (small and marginal farmer's) to participate and robustly adapt to mitigate the climate change effects on agriculture.

In the climate change scenario, agriculture needs multi-sectoral and multi-agency approach; well planned synchronised efforts for achieving sustainable agricultural productivity and nutritional security and building resilience capacity of the people and their livelihoods. Specific integrated farming system technology 'package of practices' needs to be developed depending upon the farmers' need and requirement to the specific situation. Development of information system for providing timely, accurate crop-weather advisories helps in minimizing risk and losses. Promoting/ reviving of indigenous crop varieties and reverse the loss of agro-biodiversity is essential as indigenous crops are more resilient to climate variations and farmers



have better equipped knowledge for handling them, and traditional crops generally meet the food preferences of communities. Reduction and proper utilization of waste generating from agriculture practices and post harvest stages and their utilization and value addition will reduce the carbon footprint.

Weather-based locale-specific agro-advisories, contingent crop planning, promotion of low external input technology, water budgeting, diversification for livelihood security, conservation and promotion of indigenous varieties and biodiversity need to be taken up in the respect of mixed integrated farming system are to be relooked in the newer prospective for successful adoption of farming system in the climate change scenario.

The way forward is to climate-smart sustainable integrated farming system. The healthy soil, land and ecosystem with suitable ecosystem services are the main issues, which will greatly contribute to conservation of precious resources (water, energy, soil health, etc.), while decreasing the carbon footprint in agriculture. Good quality storage facilities and marketing and value-addition of products would protect the income of small-holder producers.

# 5. WAY FORWARD

The need for diversification of farming practice is thereby needed as the income of farmers who depend solely on the produce of their traditional mono crop of rice pattern is decreasing due to limited profit margin and changed food consuming habits. Over the last two decades dietary pattern has been changed due to higher income generation, change in food habit, population explosion has also changed the supply and demand profiles of food. To meet the continuous rise in demand for food, stability of income and diverse requirements of food grains, vegetables, milk, egg and meat integrated farming systems (IFS) seems to be the feasible solution, thereby improving the nutrition of the small-scale farmers with limited available resources. Integration of different related enterprises with agriculture crops provides ways to recycle products and by-products of one component as input of another linked component which lessen the cost of production and thus raises the total income of the farm.Multiple land use through integration of crops, minor livestock with aquaculture can result in the best and optimum production from unit land area. In other words integrated farming system can be practiced as micro business by farm youthfor achieving regular income. Most of the constraints in agriculture can be removed by integration of diverse enterprises which will solve most of the existing economic and evene cological problems besides increasing productivity many-fold. Moreover, the expenditure on fertilizers also declined due to availability of a good amount of manure, which resulted into a saving of 50% expenditure on fertilizers as compared to arable farming (Tomer et al. 1982). The prospect of improved research methods for the integrated farming system is also an issue. This method should be tested in different agro-climaticzones through out the country and improve it for wide-scale adoption with low invest capital for small and marginal farmers as well as agri-enterprenuers.



#### References

- Agriculture Census. (2010) Department of Agriculture & Co-operation, Ministry of Agriculture, Government of India, New Delhi
- Anonymous (1999) Annual Report (1998-99) Fish and Prawn production in the Rice-fish diversified farming system pp69.
- Ashby JA (2001) Integrating research on food and the environment: an exit strategy from the rational fool syndrome in agricultural science. Conservation Ecology. 5(2): 20.
- Behera UK, Jha, KP and Mahapatra IC (2004) Integrated management of available resources of the small and marginal farmers for generation of income and employment in eastern India.Crop Research 27(1): 83-89
- Behera UK and Mahapatra IC (1999) Income and employment generation of small and marginal farmers through integrated farming systems. Indian Journal of Agronomy. 44(3): 431-439.
- Datta A, Nayak DR, Sinhababu DP, Adhya TK (2009). Methane and nitrous oxide emissions from an integrated rainfed rice-fish farming system of Eastern India Agriculture Ecosystem and Environment 129: 228–237.
- Faroda AS, Yadav RS and Pal RN (1978) Comparative economics of specialized dairy farming, mixed farming and arable farming. Journal of Research 8: 234-39.
- FAO (2012) FAOSTAT, http://faostat.fao.org/site/339/default. aspx
- Furuno T (2001) The Power of Duck (Integrated Rice and Duck Farming) Australia: Tagari Publications.
- Gill MS, Singh JP and Gangwar KS (2010) Integrated farming system and agriculture sustainability. Indian Journal of Agronomy 54(2): 128-39.
- Government of India (2009) Economic Survey of India, pp. 199-200. http://Indiabudget.nic.in
- Jayanthi C (2002). Sustainable Farming System and lowland farming of Tamil Nadu.IFS Adhoc scheme.Completion Report.
- Kumar Sanjeev, Singh SS, Meena MK, Shivani and Dey A (2012) Resource recycling and their management, under integrated farming system for lowlands of Bihar. Indian Journal of Agricultural Science 82 (6):504-510.
- Mahapatra IC (1992) Farming systems research challenges and opportunities. Eastern Indian Farming System Research & Extension Newsletter 6: 3–10.
- Praphan N (2001). Resilient of indigenous knowledge, fight to world crisis. Isan alternative farming network, Ubonratchathani, Thailand.
- Pullin RSV (1998) Aquaculture, Integrated Resources Management and the Environment. In: Mathias JA, Charles AT, Baotong H (Eds.), Integrated Fish Farming. Proceedings of a Workshop on Integrated Fish Farming, 11–15 October 1994, Wuxi, Jiangsu Province, China.CRC Press, Boca Raton, Florida, USA.
- Ruaysoongnern S and Suphanchaimant N (2001) land-use patterns and agricultural production systems with emphasis on changes driven by economic forces and market integration. In: Kam, S.P., Hoanh, C.T., Trebuil, G. and Hardy, B. (Eds.). Natural resource management issues in the Korat basin of northeast Thailand: An Overview. Proceedings of the Planning Workshop on Ecoregional Approaches to Natural Resource Management in the Korat Basin, Northeast Thailand: Towards Further Research Collaboration, held on 26-29 October 1999, KhonKaen, Thailand. Los Banos (Philippines): International Rice Research Institute, pp 67-77.

288



- Shamim Al Mamun, FouziaNusrat and Momota Rani Debi (2011) Integrated Farming System: Prospects in Bangladesh.J. Environ. Sci. & Nat. Resources4(2): 127-136.
- Singh K, Bohra JS, Singh, Y and Singh JP (2006) Development of farming system models for the north-eastern plain zone of Uttar Pradesh. Indian Farming 56(2): 5-11
- SinghKP, SinghSN, KumarH,KadianVSand SaxenaKK (1993) Economic analysis of different farming systems followed on small and marginal land holdings in Haryana.Haryana J.Agron. 9: 122-125.
- Sinhababu DP, Saha S and Sahu PK (2013) Performance of different fish species for controlling weeds in rainfed lowland rice field. Biocon. Sci. &Tech. 23(12): 1362–1372.
- Tomer SP, Sai Ram RK, Harika AS and Ganguly TK (1982) Comparative efficiency of dairy and mixed farming systems. Forage Research. 8:93- 98.
- Yogeesh LN, Prashant SM, Sheik Peer P and Shankar Kamble Anand (2016) Promotion of integrated farming system for enhancing the livelihood of farmers in Ballari district of Karnataka. International Journal of Science, Environment and Technology 5(5):3630-3634.⊕



# Resource Conservation Technologies under Rice-based System in Eastern India

M Shahid, AK Nayak, R Tripathi, S Mohanty, D Chatterjee, A Kumar, D Bhaduri, P Guru, S Munda, U Kumar, R Khanam, B Mondal, P Bhattacharyya, S Saha, BB Panda and PK Nayak

## SUMMARY

During the period of green revolution intensive agricultural practices has improved the yield of rice many folds, but put a big question on the sustainability of yield and soil health. Conventional rice cultivation is highly intensive in terms of water, nutrients, carbon and energy. With productivity stagnation and depletion of natural resources in northwest India due to intensive cultivation have forced to put extra efforts for increasing production and productivity in eastern India. Conventional crop establishment methods such as puddle transplanted rice require large amount of water, energy and labor, which are now becoming increasingly scarce and expensive. To ensure sustainable production and food security, it is essential to identify rice production systems with less irrigation water input. Recently, alternative resource conservation technologies (RCTs) have gained the importance to reduce the cost of cultivation and energy consumption, to sustain productivity, and to increase the profit margin of farmers. Under the changing climate scenario resource conservation technologies are viable options to shift production oriented to profit oriented sustainable farming. Improved agricultural machines have been found to be very effective on fields by reducing GHG emission. Increasing SOC in passive pool is one of the moto of climate smart agriculture. Therefore, resource conservation technologies may be considered as a realistic solution of the above-mentioned concerns.

#### **1. INTRODUCTION**

India has the biggest area (43.5 mha; 2015-16) under rice worldwide, producing 104.4 million tons of milled rice at productivity of 2.4 tons per hectare. Over the last six decades the average production per year has increased by about five times and this growth in agricultural production has come mainly from yield increase and to a lesser extent from area expansion, which is projected to decline. Furthermore, in highly intensive agricultural production areas, partial factor productivity is declining with higher input use. Therefore, future expansion in production has to come from productivity increase only through technological advancement. Post green revolution era, the crop yields have even declined in some cases, for example in the grain-producing areas of Punjab, where rice farming is characterized by intensive irrigated agriculture. It is estimated that about 137 million tons of rice would require by 2050 in India. Therefore, to sustain present status of production and to meet future food



requirement, the rice productivity has to be brought to 3.3 tons per ha from the current level.

With the evidence of productivity stagnation of rice and natural resources depletion for the past decade in the northwest part of India, the country has compelled to put extra efforts into increasing production and productivity in eastern India. A major part of eastern India receives ample rainfall (1200-1700 mm), thus providing favorable conditions for rice production. Out of the total rice growing area of the country, eastern India covers about 67% with a production proportion of 59.5% and yield of 2.15 t ha<sup>-1</sup> (Table 1), which is lower than the national average and uncertain because of its dependency on monsoon and poor management practices. Trends of decline or stagnation in productivity of rice even with the application of recommended levels of N, P and K fertilizers in intensive rice based cropping systems, reduction of soil health due to imbalance use of inorganic fertilizer, depletion of natural resources particularly, water, nutrient and labour, several economic and environmental problems

Zone	Area in percent	Rice Production	Yield (t ha <sup>-1</sup> )
East	67.0	59.5	2.15
South	15.3	18.5	2.94
North	10.8	15.7	3.53
West	5.8	4.9	2.03
Total	100.0 (43.7)*	100.0 (105.8)	2.42

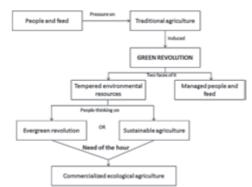
Table 1. Region wise share	of area, production, viel	ld of rice in India (2012-14).

\*Figures in parentheses indicate actual values (Area in million ha and production in million tonnes) Data source: Directorate of Economics and Statistics, Ministry of Agriculture, Government of India.

such as increasing cost of cultivation, fossil fuel burning, greenhouse gas emission, pollution of water bodies in rice production system in India in general and eastern India in particular have been manifested. Improved production technologies would help to face the challenges to produce more food at less cost and improve water

productivity, increase nutrient use efficiency and adapt the effects of climate change in lowland ecosystem in eastern India. So, there is dire need of an energy, water and labor efficient alternate system that helps to sustain soil and environmental quality, and produce more at less cost for sustainable and ecologically safe rice farming (Fig. 1).

Recently, for achieving food security the emphasis has been shifting from exploitative agriculture to conservation agriculture through the use of resource conservation







technologies in order to preserve the natural resources as well as to efficiently use the external inputs like water, chemical fertilizers and pesticides. Research efforts are focused, on the refinement of resource conservation and conservation agriculture technologies, to make the food production cost and energy efficient in order to increase the profit margins of the farmers.

Over the past decades, extreme climatic events such as extreme precipitation as well as extended drought periods or extreme temperatures have become more frequent and stronger. These extremities greatly affect the agricultural production in the region. Resource conservation technologies (RCTs) may help to adapt the production system to the effects of climate change by improving the resilience and hence making them less susceptible to aberrant climatic situations. The objective of this chapter is to discuss recent advancements of the resource conservation technologies and conservation agriculture both at international and national level with particular reference to eastern India and their potential utilization in the region.

# 2. RESOURCE CONSERVATION TECHNOLOGIES AND CONSERVATION AGRICULTURE

Resource conservation technologies (RCTs) and conservation agriculture (CA) are the two approaches which often used synonymously. However, there is distinct variation among the two. The resource conservation technologies refer to any of those practices that enhance resource- or input- use efficiency. It has a wide dimension and may include any agricultural practices that aim to conserve the natural resources and improve their use efficiencies. Direct seeding of rice which saves water, energy and labour may be considered RCTs. The varieties with high nitrogen use efficiency and minimum tillage practices which save energy, labour and improve water productivity may also be considered RCTs, as may land leveling practices that help save water. There can be many more. In contrast the term "conservation agriculture" (CA) according to the FAO, is an approach to managing agro-ecosystems for improved and sustained productivity, increased profits and food security while preserving and enhancing the resource base and the environment' (Friedrich et al. 2012). Conservation Agriculture has been based on the principles of holistic management of soil, water and other agricultural resources so as to fulfill the objective of sustainable agricultural production. CA is characterized by three major principles (FAO 2012):

- Minimal soil disturbance by direct planting through the soil cover without seedbed preparation.
- Maintenance of a permanent soil cover by mulch or growing cover crops to protect the soil surface.
- Diversifying and fitting crop rotations and associations in the case of annual crops and plant associations in the case of perennial crops.

Usually under CA, 30% surface is essentially covered either by crop residues, cover crops or biomass sourced ex-situ through agroforestry measures so as to cover



the soil surface. This surface cover helps to physically protect the soil against agents of soil degradation and provide food for the soil biota. Under the CA system, the burning or incorporation of crop residues is strictly avoided. Another important component of CA is zero tillage (ZT) technique, which restricts any kind of tillage activity and sowing of seed without or with very little soil disturbance. Due to the minimum soil disturbance in CA, soil biota and biological activities are not disturbed, that is crucial for a fertile soil which supports healthy plant growth and development. As the time passes, the soil biota acts as the agents of loosening of the soil and mixing of the soil organic matter in zero tilled fields. Crop rotation involving leguminous crop, which is another component of CA, helps to manage pest and disease problems and improve soil quality through biological nitrogen fixation and addition of organic matter.

# 3. STATUS OF RESEARCH/KNOWLEDGE - NATIONAL/ INTERNATIONAL

Globally, conservation agriculture using various RCTs is being practiced on about 125 M ha and the countries who adopted these technologies largely are USA (26.5 M ha), Argentina (25.5 M ha), Brazil (25.5 M ha), Canada (13.5 M ha) and Australia (17.0 M ha) (Bhan and Behera 2014). In India, adoption of these technologies is still in the initial phases and over the recent past, adoption of zero tillage and other technologies has expanded to cover about 1.5 M ha (Jat et al. 2012). The use of zero-till wheat in the rice-wheat system of the Indo-Gangetic plains is the major conservation agriculture based technologies being adopted. In other crops and cropping systems, the conventional agriculture based crop production systems are gradually shifting from intensive tillage to minimum/zero-tillage operations. Many countries have now policy decision to promote CA/RCT. In Europe, the European Conservation Agriculture Federation, a regional lobby group uniting national associations in UK, France, Germany, Italy, Portugal and Spain, has been founded. Resource conservation technologies are also being adapted to varying extents in countries of southeast Asia, viz. Japan, Malaysia, Indonesia, Philippines, Thailand, etc. The unique feature that triggered the widespread adoption of RCTs in many countries is community-led initiatives rather than usual research/government extension system efforts.

Most of the RCTs are already used by rice farmers of India especially northern India i.e. Punjab, Haryana etc. But adoption level of these technologies in eastern India is quite low possibly due to low level of mechanization and low availability of irrigation water. In year 2016 in south Asia the adoption level of RCT technology are direct seeded rice 22.54 %, zero till drill 11.21%, laser land levelling 6.51%, double no till 0.21%, and turbo seeder 0.10 % (D'souza and Mishra 2018). Most RCTs have been aiming at the two most crucial natural resources, water and soil. However, some of them would also affect the efficiency of other production resources and inputs such as labor and farm power or fertilizer. Some of the RCTs which are more popular and being practiced in irrigated or rice-based cropping system are outlined below.



## 3.1. Direct seeding

The shortages of labor and water, and depleting soil fertility issues are causing increasing interest in shifting from puddling and transplanting to direct seeding of rice (DSR). However, DSR is more preferred in the area with high wages and low water availability. Major constraint in the adoption of direct seeding is the high infestation of weeds, which is more difficult to manage under this system. There are in general two types of direct seeding; (1) Wet-DSR, which refers to the sowing of sprouted rice seeds either through broadcast or line sowing in puddled soil; and (2) Dry-DSR, under which dry rice seeds are sown either through drilling or broadcasting in the field that was prepared either by dry tillage or zero tillage or on a raised bed. One more category of DSR is referred as water seeding where sprouted rice seeds are broadcasted in standing water.

Wet-DSR is mostly practiced to manage the shortage of labour; however, increasing scarcities of water, prompted to develop and adopt Dry-DSR. As far as yield is concerned, differential response is observed for direct seeding of rice. In India, yields were significantly lower (9.2–28.5%) in Dry-DSR than in conventional tillage transplanting rice (CT-TPR). In Pakistan, yields of both Wet- and Dry-DSR were 12.7–21.0% lower than CT-TPR. In Bangladesh and the Philippines, yields of CT-wet-DSR were higher (8.6–18.5%) than those of CT-TPR, whereas in Nepal, Thailand, Combodia and Laos, yields were similar to those of CT-TPR (Kumar and Ladha 2011). Studies comparing CH<sub>4</sub> emissions from different tillage and crop establishment methods but with similar water management (continuous flooding/mid-season drainage/intermittent irrigation) in rice revealed that CH<sub>4</sub> emissions were lower with Wet- or Dry-DSR than with CT-TPR (Kumar and Ladha 2011). The reported reduction in CH<sub>4</sub> emissions was higher in Dry-DSR than in Wet-DSR.

#### 3.2. Bed planting

Bed planting is a system of crop production where the crop is grown on raised beds, and furrows in between the beds are used for irrigating the crops. This system has advantages of irrigation water saving, enhanced fertilizer use efficiency, better weed management, and a reduced seed rate. Under bed planting system, significant quantity of water is saved due to reduced evaporation and better distribution. Additionally, the rooting environment is modified and aeration of the bed zone is improved as compared with flat planting. Different type of bed plantings can be used under different situations such as raised-bed transplanted rice, raised-bed drill-seeded rice and permanent (double) bed-planted rice.

#### 3.3. Minimum and zero tillage

Conventionally, tillage is done for the loosening of soil, preparation of seedbed for good and uniform seed germination, management of weeds and incorporation of crops left over, manures and fertilizers into the soil. While intensive tillage of the soil has some immediate advantage, there are negative effects of continuous tillage on soil quality which become more evident over the longer period. Soil organic matter



(SOM) status is generally considered as the most dominating parameter for the determination of soil quality and there is ample evidence of declining SOM with tillage as compared to relatively undisturbed soil, which affects soil aggregation, water availability, soil aeration, nutrient availability and soil microbial activity. Frequent tillage tends to develop hardpan at the bottom of the plough layer which can restrict water infiltration and root penetration. In recent years, considerable modifications have taken place in tillage operations and several new concepts have been introduced namely minimum tillage and zero tillage.

Minimum tillage involves considerable soil disturbance, though to a much lesser extent than that associated with conventional tillage. It is aimed at reducing tillage to the minimum necessary for ensuring a good seedbed, rapid germination, a satisfactory stand and favourable growing conditions. Zero tillage (ZT) implies the sowing of seed with the use of such tillage implements that open a narrow slit to drop the seed and does not turn over the soil during the operation. Due to skipping of the intensive ploughing that involves 3-4 tillage operations in traditional approaches, cost of production is reduced and timely planting of subsequent crop is ensured.

Evidence on yield effects of zero tillage over conventional tillage is highly variable and it was observed that zero tillage in combination with mulching resulted in initial yield decline, and later on increase over the subsequent period (Baudron et al. 2011), eventually exceeding yields in conventional tillage-based agriculture (Rusinamhodzi et al. 2011). Other advantages of ZT over conventional tillage include better soil health, energy saving and buildup of organic carbon. Zero tillage helps to mitigate the effects of climate change through soil organic carbon sequestration and reduced greenhouse gases emission. Various minimum and zero tillage operations used under RCTs are minimum-till (non-puddled) transplanted rice, minimum-till (non-puddled) dry drill-seeded rice, minimum-till drill-seeded rice+sesbania, zero-till (nonpuddled) transplanted rice, zero-till drill-seeded rice, zero-till drill-seeded rice+sesbania and double zero-till drill seeded rice.

## 3.4. Laser-assisted land leveling (LASER-level)

For surface-irrigated areas, a properly leveled surface with the required inclination according to the irrigation method is absolutely essential. In the traditional method of land leveling using eyesight, particularly on larger plots, getting accurate results are difficult which lead to increased irrigation times, superfluous and inefficient water use. Laser leveling is a process of flattening the land surface ( $\pm 2$  cm) from its normal elevation by using laser equipped drag buckets to achieve precision in land leveling. By following this practice, a reduction in irrigation water and increase in yield of wheat crop has been observed.

# 3.5. Alternate wetting and drying irrigation method

Alternate wetting and drying (AWD) is a method of water application during rice cultivation which can be followed to reduce the water consumption in irrigated fields. In this method, rice fields are alternately flooded and dried on frequent interval. The



drying period of the soil in AWD vary according to the type of soil and the cultivar. Alternate wetting and drying has been commonly used as a technology to save water in many parts of the world for over a decade, however, unproductive water losses could not be totally avoided due to the submergence of the field during irrigation periods. The alternate wetting and drying technology not only saves water but can greatly reduce emissions of methane.

#### 3.6. System of rice intensification

The system of rice intensification (SRI) is a knowledge-based low-external input technology which is developed in the 1980s in Madagascar and it benefit farmers with small landholdings by giving higher yields without any harmful impact on natural resources. It helps the rice farmers in increasing yield and provides some other benefits such as saving of water and other inputs, if this is done in conjunction with other changes in how they manage the plants, soil and nutrients. The main attributes of this technology are transplanting of single young seedlings in a square pattern with wider spacing, using organic manures and following alternate wetting and drying irrigation method while keeping the rice soil moist during the vegetative growth phase. This practice led to significant phenotypic changes in plant structure and function and in yield and yield attributing characters. System of rice intensification increased yields substantially (50-100% or more), while consuming only about half as much water as conventional (Uphoff et al. 2011), whilst not needing the purchase of additional external inputs. This technology has been proven to be resilient against extreme weather events, pests, and diseases because of better plant vigor and root structure. In recent years, many modifications in SRI has been noticed in different parts of the world and it is asserted that the package of possible practices under SRI have to be adapted to local conditions (Stoop 2011). Moreover, somewhat higher labour requirement for SRI, poses the challenge to researchers and policymakers concerned with the promotion of water saving rice technologies.

#### 3.7. Cover crops and crop residues

One of the fundamental principles of the CA is to keep the soil surface covered. This can be achieved either through growing cover crops or retention of crop residues of previous crop on the soil surface. Cover crops are generally grown in between two main crops to fill the gap period of harvesting one crop and establishing the next in the fields where gap is too long. The vegetative biomass of cover crops helps to protect the soil against the impacts of raindrops, provide shading of soil and preserve the moisture loss. They may also benefit the main crops by providing mineral nutrition through nitrogen fixation (legumes) and mineralization of other nutrients. Cover crops may generate additional income through production of additional grain for human food or extra fodder resources. There are different crop options such as grains, legumes, root crops and oil crops that can be used as vegetative cover.

Crop residues are that portion of the crop which is left in the field after harvest, or that part of the crop which is not used locally or traded commercially or discarded during processing. More than 352 Mt of crop residues from various crops are produced



annually of which major quantity is contributed by rice and wheat (Singh and Sidhu 2014). At many parts of the country especially the northern part, in situ burning of the crop residues is a major concern that not only pollutes the environment but also cause a loss of about 6 Mt of major nutrients. If managed properly, the crop residues may become important source of nutrients and can also maintain or enhance soil chemical, physical and biological properties along with preventing land degradation. Some studies have found that recycling of crop residues in rice-wheat system increased the rice as well as wheat yields by 13 and 8% and energy efficiency by 13 and 6%, respectively with a decrease in cost of production, compared to residue burning (PDFSR 2011). The major concerns in utilization of crop residues are the slow rate of decomposition due to high C: N ratio of rice and wheat residues; obstruction during tillage and sowing operation; and the increased incidence of disease limit its use.

#### 3.8. Crop rotation and cropping system

Crop rotation is considered an essential component of the conservation agriculture for achieving higher diversity in plant production. Growing diverse crops in sequence not only maintain the higher soil microbial diversity but also help to explore the different soil layers and recycle the nutrients that have been leached down to deeper layers. Crop rotations involving legumes create favourable soil condition for the proliferation of diverse soil biota, reduce input costs due to nitrogen fixation, provide better distribution of water and nutrients through soil profile and help to manage pests and diseases. Intensification of cropping systems such as increased number of crops per year, double cropping, and addition of cover crops can increase soil C storage under no-tillage (Luo et al. 2010). Efficient cropping sequences can contribute to a great extent to sustain agricultural production.

#### 3.9. Nutrient Management under RCT/CA

Eastern India is more agriculturally productive due to fertile soil, rainfall pattern and plenty of available irrigation water, however more vulnerable to climate change. Nutrient management is one of the prime options that are responsible for sustaining crop productivity as well as maintaining soil quality. Under the periphery of resource conservation technology, nutrient input in soil can be supplemented through: integrated nutrient management (INM) and organic nutrient management (ONM). Both the options can be suitable to improve the potential of yield under rice based system of a specific agro-ecological region depending on the soil type, soil fertility status and crop history.

An integrated nutrient management (INM) ideally combines both inorganic and organic sources of nutrients in a balanced way. While inorganic nutrient forms readily supplies plant essential ions and ensures better crop productivity; the organic forms of nutrients, on the other hand, increase the nutrient use efficiency in soil and reduces the chances of soil pollution due to excess use of factory generated chemical fertilizers. Hence, the combination of both is often preferred for rice based farming system, especially in the eastern Indian condition.



The sole organic nutrient management (ONM) is not as popular among the farming communities of lowland rice system because of the uncertainty of the yield performance of different rice varieties. But considering the sustenance of soil health, the locally-available and cost-friendly investments of organic manures are sometimes preferred.

## 3.10. Weed management under RCT/CA

Economic factors and technology options have led to the change in rice establishment method from traditional transplanting system to direct-seeding in Asian rice systems over past few years. Weed infestation, however continues to be a major bottleneck in this method because of simultaneous emergence of rice and weeds and absence of standing water during early stages of crop growth to suppress germinating weeds. Dry seeding of rice with zero tillage may further aggravate the weeds problem because no tilling concentrates the weed seedbanks in the top layer of the soil that results in a higher proportion of seed germination compared with conventional tillage (Gallandt et al. 2004). Tillage also affects other soil growth factors such as temperature, moisture, aeration and nutrients which affect weed infestation (El-Titi 2003).

Successful cultivation of direct-seeded rice, particularly under zero-tillage system requires intensive use of herbicides. A variety of herbicides have been screened and found effective for pre-plant/burn down, pre-emergence, and post-emergence weed control in dry direct drill-seeded rice systems (Kumar and Ladha 2011). Because of their high dormancy, the weeds of some weed species keep germinating throughout the growing season. Pre- and post-emergence herbicides are imperative to keep weeds under check (Kamboj et al. 2012). This practice of using pre- and post-emergence herbicides is leading to pesticide load in the environment which is highly undesirable. Therefore, cultivation practices should be evolved and designed in such a way that the dual issues of weed management and rational use of pesticides are tackled simultaneously.

## 3.11. Machineries used under RCT/CA

Improved agricultural machines have been found to be very effective on fields by reducing GHG emission under precise land levelling, no tillage or zero tillage, sowing machinery and subsequent efficient machinery for intercultural, harvesting and post harvesting operations. Some of the machines that are commonly used are outlined below.

**3.11.1. Tractor drawn laser land leveler:** The tractor drawn laser land leveller is used for micro-levelling of fields and filling loose soil from higher elevation place to lower place. Precision leveling increase yield by 20-30% and helps in control of weeds and pests both in wet lands as well as up lands. This machine includes drag scraper, laser transmitter, laser receiver, hydraulic control system, control panel and tripods stand.

**3.11.2. Tractor drawn rotavator:** Rotavator is an effective modern implement suitable for all types and textures of soil. It effectively and economically replaces the combined functions of cultivator, disc harrow, leveler and manual labour. It can be effectively used as a puddler. It produces green manure by cutting roots/weeds in small fragments

and mixing with soil. It creates better aeration and rapid germination of seeds. Preparatory time of seedbed preparation between two crops reduces, hence it provide opportunities for growing of second crop.

**3.11.3. Direct rice seeder:** Rice can be directly seeded either through dry or wet (pregerminated) seeding. Dry seeding of rice can be done by drilling the seed into a fine seedbed with a seed drill. Wet seeding is done through the use of drum seeder in leveled puddled fields. Direct sowing of rice solve the problem of labour and water scarcity. It gives saving of 12-35% of manpower and also reduction in methane emission. Direct rice seeding methods results in higher economic return than conventional transplanting.

**3.11.4. Happy seeder:** The Happy seeder is used for direct drilling of rabi crop in to a combine harvested field (without straw removal/burning) in a single operation. The rotating blades cut only that part of straw which is coming just in front of furrow openers. These cutting blades are operated by PTO drive of tractor. It consists of two units- one straw management unit and other sowing unit. The happy seeder cuts, lifts and place the standing stubble and loose straw and sows the field in one operational pass of the machine.

**3.11.5. Tractor drawn zero till seed drill:** This machine is used to sow the crop directly into the uncultivated field just after the harvest of previous crop (rice) by eliminating the tillage operation. It consists of fluted rollers for metering of seed and fertilizer. The ground drive wheel supplies power through sprocket and chain for metering of seed and fertilizer. The operation of zero till drill is energy efficient and cost effective and it ensure timeliness of planting by avoiding repeated tillage operations. It is suitable for sowing of wheat, gram, peas, soybean and linseed. The machine is recommended for adoption by farmers for timely planting of crops viz. wheat, gram, peas, soybean, linseed etc. during the brief turnaround time after harvest of rice to increase the productivity and profitability of subsequent crop.

**3.11.6. Straw reaper:** It is a machine that cut threshes and cleans the leftover straw in one operation. The stalks are conveyed into the machine by auger and guide drum and reached to the threshing cylinder, where it cuts into small pieces. The short fragments of stalks fall through the bars of the concave and the straw is collected in an attached trolley and collected.

# 4. STATUS OF RESEARCH AT ICAR-NATIONAL RICE RESEARCH INSTITUTE

ICAR-National Rice Research Institute (NRRI) has been in the forefront of developing and refining resource conservation technologies for lowland rice in eastern India. Many of the earlier works of the ICAR-NRRI was focused on improving the use efficiency of the natural resources, increasing productivity of rice and reducing GHG emission along with building up of carbon by developing the technologies related to direct seeding, system of rice intensification, cropping system research involving



legume crops, rice residue management, minimum tillage and zero tillage both under transplanted and direct seeded conditions. The institute also worked upon the designing and development of farm equipment for small and medium farmers related to rice sowing and weeding. Some of the major findings are discussed below.

Saha (2005) reported that pre-emergence application of pyrazosulfuron ethyl + molinate at 1.0 kg ha<sup>-1</sup> supplemented with one hand weeding at 50 days after rice sowing maintained a lower crop weed competition from the seeding of the crop till maturity and registered the lowest weed density (3.2 m<sup>-2</sup>) in direct seeded rainfed lowland rice. Moorthy and Saha (2003) reported that single application of butachlor (2.0 kg ha<sup>-1</sup>), butachlor+safener (2.0 kg ha<sup>-1</sup>), quinclorac (1.0 kg ha<sup>-1</sup>) and fluchloralin (1.0 kg ha<sup>-1</sup>) controlled weeds effectively and weed control efficiencies ranged from 63.5 to 71.6 per cent in rainfed lowland direct seeded rice. A supplementary hand weeding controlled the weeds substantially as evidenced by comparable dry weight of weeds recorded in these treatments with that of weed free check and higher weed control efficiencies (82.1 to 85.3%).

Field experiment conducted to standardize the age of seedlings with different crop densities for realization of higher yield in SRI indicated that 14-days old seedlings planted at 16 hills m<sup>-2</sup> gave significantly higher grain yield than conventional practice (CRRI 2008). The yield potential of rice under SRI with different plant geometry and age of seedlings was studied with the rice hybrid Ajay. Higher grain yield of 6.43 t/ha was recorded with 8 days old seedlings planted at 25 cm x 25 cm spacing which was 5.75% and 14.4% higher than 14 and 21 days old seedlings at same spacing ( Lal et al. 2016).

In a field experiment it was observed that wet tillage recorded significant yield advantage over the dry direct seeded practice, however, tillage depth had no significant effect on yield. During dry season, greengram cv. PDM 54 produced 48% higher yield in the plots where dry direct sown rice was grown in the preceding season (CRRI 2012).

A study conducted at ICAR-NRRI on rice residue management revealed that substitution of chemical N (25%) with crop residue to provide 60 kg N/ha gave comparable grain and straw yields as that of chemical N. Superimposition of 60 kg chemical N ha<sup>-1</sup> (urea) on 2.5 t ha<sup>-1</sup> crop residue increased the grain and straw yield of rice over sole application of urea (CRRI 2011).

Field experiment was conducted with different resource conservation technologies (RCTs) viz. minimum tillage, green manuring, brown manuring, wet direct seeding of rice, zero tilled dry direct seeded rice and paired row dry direct seeded rice. The grain yield varied in the range of 4.08 to 4.98 t/ha and the highest yield was obtained in the paired row dry direct seeded rice with dhaincha. The input and output energy varied in the range of 17.9-31.1 and 147.1- 190 GJ ha<sup>-1</sup>, respectively with highest input energy was in paired row dry direct seeded rice dhaincha. The energy ratio (output/input) was also found highest in the paired row rice with dhaincha treatment. There was significant



amount of energy savings and net C gain in paired row dry direct seeded rice with green manuring treatment compared to the rest of the treatments in this study (NRRI 2015).

A best bet climate-smart resource conservation technology (CRCT) involving the 'package of primary land preparation with mould board plough (once in three year) followed by soil pulverization by cultivator, dry direct seeding of rice and dhaincha in paired row (15 cm spacing) with seed drill, dhaincha incorporation by cono-weeder at 25 DAS (alternatively knock down of dhaincha by 2,4-D, if standing water is not available), 75% RDF of N and full doses of P and K as basal, customized leaf colour chart (CLCC) based N application in two splits and mechanical harvesting by reaper' was developed and validated in the farmers field and recommended for higher yield, low energy and environment sustainability (Bhattacharyya et al. 2014).

In an experiment conducted at ICAR-National Rice Research Institute, Cuttack it was observed that SOC stock was significantly higher by 5.3-9.7% in zero tillage transplanted rice as compared to conventional rice cultivation (Dash et al. 2017). Labile carbon pools are highly sensitive to cultivation practices, on the contrary total carbon content does not change much. Therefore, carbon dynamics can be understood by observing the changes in labile carbon pools under various RCT practices. Among various RCT, residue incorporation and green manuring, in general, showed higher quantity of readily mineralizable C, microbial biomass C, water soluble C, acid hydrolysable carbohydrate and permanganate oxidizable C compared to other techniques (Dash et al. 2017).

For small and medium farmers rice sowing and weeding equipment were developed by ICAR-NRRI Cuttack. For sowing of pre-germinated seeds manual operated drum seeders having four rows, six rows and power operated eight row seeder was developed. Power tiller operated sowing machinery were developed which can be used in sowing of rabi crop in rice crop residues. Manual and power operated weeders were developed for weeding in wet land rice and dry land subsequent crops.

# 5. KNOWLEDGE GAPS

Intensive agriculture takes its toll on natural resources and on the environment. This means that farmers have to produce more from less and deteriorating resources. Keeping this in mind, the sustainability of rice farming under existing conditions in eastern India is not assured without special efforts.

- Improper management of soil resource leads to degradation of the soil, resulting in poor productivity. Soil quality assessment is, therefore, an important factor for sustainable use of soil resources.
- Under zero tillage, the weeds emerge during the fallow just before the crop season which makes the sowing difficult. Under this scenario, submergence of soil before seeding or transplanting has its own advantages, viz., restricting the weed seed germination under anaerobic condition, and decaying of emerged weeds. Intensive



research is required to be done on water seeding or mechanical transplanting in standing water.

- The success of resource conservation technologies in eastern India depend on two critical elements viz. residue retention on surface and weed control. Since residues are generally used as fodder in rainfed, there is a need to determine the minimum residue that can be retained without affecting the crop-livestock system.
- Any reduction in tillage intensity and frequency, poses serious concerns with regard to weed management. Weed control strategies have remained challenging for agricultural lands being switched to conservation tillage practices. Weed management under zero tillage has been reported in many crops including cereals, however, no comprehensive weed management strategy presently exists in unpuddled transplanted rice.

# 6. RESEARCH AND DEVELOPMENT NEEDS

It is claimed that the full benefits of CA can be availed when all the three principles (minimum soil disturbance, permanent soil cover through crop residues or cover crops, and crop rotations) are followed i.e. the concept of CA is fulfilled. However, there is probably no scientific report available on rice in India that proves this fact. A thorough investigation on different components of conservation agriculture either alone or in combination may help in the assessment of individual and combined benefits that will lead to appropriately adjust the requirement at specific sites.

In eastern India, dominant rice ecology is the rainfed lowland. Under this ecology moisture availability is enough and in general nutrient status is good, and if managed properly it can support short duration rabi crop. Under such ecosystem, short-duration pulse crop can be grown in sequence to the kharif rice crop. Short duration pulses which have much lesser water requirement and require shorter duration can thrive well under harsh climate and fragile ecosystems on one hand and can help to achieve household food and nutritional security on the other, offer great promise towards diversification of cropping systems.

In rice, it has been observed that strategies to reduce emissions of  $N_2O$  often lead to an increase in emissions of  $CH_4$ . There is trade-off between these two gases. Both gases have different global warming potential (GWP), hence our approach must be focused on reduction of  $N_2O$  emission, because  $N_2O$  is having more GWP than  $CH_4$ . The RCTs measures with lesser emission of  $N_2O$  need to be recommended for rice based systems.

Some strides have been made for herbicide screening in DSR, however, research is needed to evaluate the efficacy of different herbicides which can be effective for weed control under zero-till systems. More research on weed management under minimum tillage in a cropping system perspective is needed. Identification of critical thresholds of tillage for various rainfall, soil and cropping systems, such that the main objectives of rainwater conservation are not compromised. This will balance the need for conserving soil and capture rainwater in the profile.

Ecosystem services are crucial for the sustainable supply of food and fibre. Unsustainable agricultural practices pose great threat to worldwide food supply due to declining ability of the agricultural ecosystems to provide ecosystem services. Assessment of ecosystem services under different RCT for long-term sustainability of agro- ecosystems and their ability to provide increased production without deteriorating the ecosystem services need to be done. With the accumulating evidence on climate change, there has been interest in examining the greenhouse gas contribution of production practices and products using life cycle assessment approach. The sole application of mineral fertilizers could not maintain SOC stock while application of organics proved essential for long-term sustainability of the rice based system. Combination of rice residue and green manuring is adoptable soil amendment option to sequester the soil organic C, yield sustainability and minimizing GHG emission. Study on sustainability, C sequestration and ecosystem functioning for rice-based system under organic nutrient management can help in understanding the ecological footprint of the organic nutrient management. Application of optimum quantities of inputs (mainly renewable energy) and utilizing positive synergies between the agricultural crops will minimize the wastes of nutrients, water and locally available organic wastes is the key principles for achieving sustainability. Strategies for low energy inputs and synergy, conservation agriculture practices will minimize the environmental impacts, making rice farming sustainable and eco-efficient. Crop residue management is a challenge in rice farming and possible ways need to be explored for fast decomposition of paddy straw.

Availability of machinery/equipment for promotion of RCTs is a prerequisite for achieving targets of agricultural production. More user friendly technology need to be developed for direct drilling of rabi crop in heavy paddy residue condition. Development of specialised machineries such as paired row rice-daincha seeder is required for doing the simultaneous operations in one go and save energy and labour. Farm implements needed for seed and fertilizer placement simultaneously for ensuring optimum plant stand, early seedling vigour in rainfed crops under minimum tillage. There is a need to enhance the accessibility of smallholders to zero-till knowledge, herbicides, and zero-till planters.

# 7. WAY FORWARD

Resource conservation technologies and conservation agriculture offer a new paradigm for agricultural production different from earlier systems, which mainly intended to achieve a specific production targets. Locally adapted RCTs appropriate to resource availability of farmers and the biophysical condition hold potential to improve management of natural resources and provide sustainable increases in productivity. From the long-term field experiments, which were started at the beginning of the green revolution era for developing nutrient management strategies and understanding the nutrient mining, valuable information have been received to develop future strategies. Appropriate long-term monitoring has to be continued, and be relevant to future changes in tillage and water management practices. In addition,



benefits of changes in the tillage system and stubble management to the soil ecosystem need to be better understood. Zero-till, permanent bed-planting systems and new non-puddled rice establishment techniques coupled with laser land levelling can go a long way to increasing the use efficiency of this vital natural resource. Resourceconserving technologies applied in isolation have advantages and disadvantages. They are not universally applicable as the problems can sometimes outweigh the benefits. However, by combining different resource-conserving technologies, synergies can be created to eliminate the disadvantages of single technologies and accumulate the benefits.

The increased use of chemical fertilizers to increase the production of food and fibre is a cause of concern and it was realized that the soils which receive mineral nutrients only through chemical fertilizers are showing declining or stagnating productivity even being supplied with sufficient nutrients. In recent years, the agricultural production system is facing the challenges of energy crisis, high fertilizer cost and low purchasing power of the farming community which necessitates rethinking on the alternatives. Unlike chemical fertilizers, organic manure and biofertilizers are available locally at cheaper rates. They enhance crop yields per unit of applied nutrients and provide a better physical, chemical and microbial environment that is more conducive to higher productivity. The available quantity of animal manure and crop residues cannot meet the country's requirements for crop production. Therefore, maximizing the usage of organic waste and combining it with chemical fertilizers and bio-fertilizers in the form of integrated manure appears to be the best alternative.

Mitigation options in the rice based cropping system may individually be of limited scope, but they may achieve a discernable composite effect when implemented in coordinated fashion. Mitigation programs will rely on win–win opportunities when emissions can be reduced with another concomitant benefit such as higher yields, less fertilizer, and water needs etc. Targeting one individual gas alone seems inappropriate due to tradeoff effects in the emissions of  $CH_4$ ,  $N_2O$ , and  $CO_2$ . More research is needed to combine geographic information, emission models, yield models and socio-economic information to devise site-specific packages of mitigation technologies. Many studies conducted on RCT shows reduction in cost of cultivation, reduced incidence of weeds, saving in water and nutrient, increased yields and environmental benefits. Still RCTs are not popular among farmers of eastern India. These technologies need to be popularizing among farmers with the help of extension departments.

#### References

- Baudron F, Tittonell P, Corbeels M, Letourmya P and Giller KE (2011) Comparative performance of conservation agriculture and current smallholder farming practices in semi-arid Zimbabwe. Field Crops Research 132:117-128.
- Bhan S and Behera UK (2014) Conservation agriculture in India Problems, prospects and policy issues. International Soil and Water Conservation Research 2(4):1-12.



- Bhattacharyya P, Nayak AK, Din M, Lal B, Raja R, Tripathi R, Mohanty S, Shahid Mohammad, Kumar A, Panda BB, Munda S, Gautam P and Mohapatra T (2014) Climate-smart resource conservation technology (CRCT) for lowland rice ecologies in eastern India. CRRI research bulletin No. 8. ICAR-Central Rice Research Institute, Cuttack, pp. 1-28.
- CRRI (2008) Annual Report 2007-08. Central Rice Research Institute, Cuttack, p. 58.
- CRRI (2011) Annual Report 2010-11. Central Rice Research Institute, Cuttack, p. 21.
- CRRI (2012) Annual Report 2011-12. Central Rice Research Institute, Cuttack, p. 66.
- D'souza A and Mishra AK (2018). Adoption and abandonment of partial conservation technologies in developing economies: The case of south Asia. Land Use Policy 70:221-223.
- Dash PK, Bhattacharyya P, Shahid M, Roy KS, Swain CK, Tripathi R and Nayak AK (2017) Low carbon resource conservation techniques for energy savings, carbon gain and lowering GHGs emission in lowland transplanted rice. Soil and Tillage Research 174:45–57.
- El-Titi A (Ed.) (2003) Soil tillage in agroecosystems. CRC Press, Boca Raton.
- FAO (2012) What is conservation agriculture. Available at: http://www.fao.org/ag/ca/1a.html (accessed 21, December 2017).
- Friedrich T, Derpsch R and Kassam A (2012) Overview of the global spread of conservation agriculture. Field Actions Science Reports 6:1–7.
- Gallandt ER, Fuerst EP and Kennedy AC (2004) Effect of tillage, fungicide seed treatment, and soil fumigation on seed bank dynamics of wild oat (Avena fatua). Weed Science 52:597-604.
- Jat ML, Malik RK, Saharawat YS, Gupta R, Bhag M, Paroda R (2012) Proceedings of regional dialogue on conservation agricultural in south Asia, New Delhi, India, APAARI, CIMMYT, ICAR 31.
- Kamboj BR, Kamboj BR, Kumar A, Bishnoi DK, Singla K, Kumar V, Jat ML, Chaudhary N, Jat HS, Gosain DK, Khippal A, Garg R, Lathwal OP, Goyal SP, Goyal NK, Yadav A, Malik DS, Mishra A and Bhatia R (2012) Direct seeded rice technology in western Indo-Gangetic Plains of India: CSISA experiences. CSISA Technical Bulletin 2012/1. CSISA, IRRI, CIMMYT, New Delhi (India).
- Kumar V and Ladha JK (2011) Direct seeding of rice: Recent developments and future research needs. Advances in Agronomy 111:297–413.
- Lal B, Nayak AK, Gautam P, Tripathi R, Shahid M, Panda BB, Bhattacharyya P and Rao KS (2016) System of rice intensification: A critical analysis. Research Bulletin No. 9. ICAR-National Rice Research Institute, Cuttack, Odisha, 753006, India, p. 52.
- Luo Z, Wang E and Sun OJ (2010) Can no-tillage stimulate carbon sequestration in agricultural soils? A meta-analysis of paired experiments. Agriculture Ecosystem and Environment 39:224-231.
- Moorthy BTS and Saha S (2003) Relative performance of herbicides alone and in combination with hand weeding in rainfed lowland direct seeded rice. Indian Journal of Weed Science 35(3&4):268-70.
- NRRI (2015) Annual Report 2014-15. ICAR-National Rice Research Institute, Cuttack, pp. 86-89.
- PDFSR (2011) Project Directorate for Farming Systems Research. Annual Report 2011-12.
- Rusinamhodzi L, Corbeels M, van Wijk MT, Rufino MC, Nyamangara J and Giller KE (2011) A meta-analysis of long-term effects of conservation agriculture on maize grain yield under rain-fed conditions. Agronomy for Sustainable Development 31:657-673.



- Saha S (2005) Evaluation of some new herbicides alone or in combination with hand weeding in direct seeded rainfed lowland rice. Indian Journal of Weed Science 37(1&2):103-104.
- Singh Y and Sidhu HS (2014) Management of cereal crop residues for sustainable rice-wheat production system in the Indo-Gangetic plains of India. Proceedings of Indian National Science Academy 80:95–114.
- Srivastava P, Singh R, Tripathi S and Raghubanshi AS (2016) An urgent need for sustainable thinking in agriculture An Indian scenario. Ecological Indicators 67:611–622.
- Stoop W (2011) The scientific case of the system of rice intensification and its relevance for sustainable crop intensification. Internatinal Journal of Agricultural Sustainability 9:443-445.
- Uphoff N, Kassam A and Harwood R (2011) SRI as a methodology for raising crop and water productivity: productive adaptations in rice agronomy and irrigation water management. Paddy and Water Environment 9:3-11.♥



# **Dynamics and Management of Weeds in Rice**

# S Saha, S Munda, BC Patra, T Adak, BS Satapathy, P Paneerselvam, P Guru, NT Borkar and S Chatterjee

## SUMMARY

Weeds are serious problems for farmers of all hues and rice growing is no exception. Problems associated with weed management in rice are mounting dramatically during last few decades due to rapid changes in cultural practices of rice farming and also because of reduced availability of affordable labour and shortage of water. Changes in cultural practices viz., mechanized tillage, crop establishment by direct seeding, increased herbicide use, variable water availability, mechanized harvesting, rice-rice cropping sequence etc. led to shift from relatively easy-to-control sedges and broadleaved weeds to more difficult-to-control grassy weeds including weedy rice. New management strategies are required as no single method can solve all the problems of weeds in rice cultivation. Manual weeding 2-3 times in a season by engaging more than 100 person days ha-1 involves huge cost in weed control. Selection of suitable rice varieties with proper management practices should be integrated with direct control measures viz., mechanical weed control by using motorized weeder or by application of safest herbicide with broad spectrum to reduce the cost on weed management practices. The overall objective of modern weed management approaches is to reduce the degree of direct control inputs. Therefore, further research is needed for breeding weed competitive rice cultivars, herbicide tolerant rice. Also development of power weeder with high operation efficiency is highly important. Along with these technologies, emphasis on development and standardization of new and safe herbicides should be given to make rice cultivation more profitable.

# **1. INTRODUCTION**

Weeds are undoubtedly a major biotic constraint to rice production, causing 33% of total yield losses in comparison to insects (26%) and diseases (20%). Extent of dominance of weed is dependent on prevailing agro-climatic conditions, soil types, water management, crop establishment practices, weed seed bank in soil and cropping system adopted in different rice ecologies. Depending upon various factors, the yield loss varies from 30% in irrigated to 70% in rainfed uplands (Saha and Rao 2011). Beside this, weeds interfere with rice growth by competing for light, nutrients, water and space and by creating a favourable habitat for the growth of various harmful organisms such as insects and pathogens. Problems associated with rice weeds are mounting dramatically due to changes in rice production systems in response to changing climate and declining accessibility of labour and water. Weeds are dynamic and new weeds keep emerging over a period of time owing to change in cropping pattern and, practices of crop cultivation. Changing climate may also lead to weed shifts, faster spread of invasive species and more competition to crops from weeds.



Change in traits and growth behavior in response to climate change is expected to make weed scenario more complex.

Access to supplementary irrigation has enabled crop establishment by direct seeding in non-puddled, non flooded fields under dry condition and lowland rice environment with limited water (Singh et al. 2006). Water and labour scarcity is also pushing the farmers to opt for direct seeded systems. These non-puddled, non flooded systems are threatened by heavy weed infestation. The absence of a seedling-size advantage between rice and weed seedlings, as both emerge simultaneously, can cause grain yield losses of 50–91%. Thus, weeds are the most severe constraints to direct sown aerobic rice systems (Rao et al. 2007). The key to success of these systems is efficient weed control techniques. Recently, weedy rice is emerging as another serious threat to direct seeded systems. The spread of weedy rice became significant all over the world mainly after the shift of rice cultivation from transplanting to direct seeding. For farmers, weedy rice is a difficult-to-control weed/ plant as strategy for its management is non-existent, and still remains elusive in non flooded aerobic situations (Saha et. al. 2014). The conception of herbicide tolerant (HT) rice may offer rice farmers a vital tool for controlling difficult to control grasses and mixed population of weeds. It can also help to control wild and other weedy rice species and provide an alternative tool for the management of those weeds that have already evolved resistance to particular herbicides. It also makes way for the replacement of some of the commonly used selective herbicides by new non-selective, environmentally safe herbicides.

Traditionally, manual weeding is done 2-3 times in a season (more than 190 person days/ha used) which involves huge cost in weed control. Additionally, seedlings of grassy weeds (e.g., Echinochloa spp.) look similar to rice seedlings and it makes hand/ manual weeding more tedious and difficult. Therefore, use of herbicides (and/ or bio-inoculants), or using machines (mechanical weed control) are considered as alternative/supplement to manual-weeding and most economical way to manage weeds. New safer herbicides need to be formulated and standardized for broad spectrum weed control in rice under different situations. However, use of herbicides is deemed with its own challenges like environmental pollution and herbicide resistance. In absence of strict guidelines and its implementation, herbicides are being used in excess, which cause water pollution through run-off, and negatively affect the soil by affecting the microbes. Weed resistance to the herbicides used in rice is a relatively new event. Since 1980s', with the introduction of sulfonylurea herbicides, several weed species have evolved resistance to herbicides due to continuous use of same herbicide in the same field. Even multiple resistances (the resistance to more than one type of herbicide action) have evolved in some cases. Low cost single row and modified two row self-propelled power weeder may serve as an alternative to herbicide with less drudgery. The machine has been designed and tested at ICAR-NRRI with 28-30% plant damage (CRRI Annual Report 2013-14). Further research is required to design efficient implements (weeder) to cut down the energy and cost incurred for manual weed control.



An important factor of modern weed management strategy – is not to rely too heavily on any one tactic. Current weed management technology consists of integration of appropriate crop husbandry (agronomic practices) along with direct cultural and chemical methods (Saha and Rao 2011). Thus, an integrated approach involving appropriate crop husbandry along with some direct weed management practices viz., cultivation of weed competitive varieties, selection of herbicides and farm mechanization. This chapter on weed management would give an insight on the possibilities to tackle the threat called 'weeds' with the existing knowledge and contemplate on what could be done further based on existing technologies. This chapter also explores the challenges and responsibilities that lie ahead in future rice production vis-a-vis weed management.

# 2. STATUS OF RESEARCH/KNOWLEDGE

#### 2.1. Rice crop-weed interference

Among different categories of weed flora, grassy weeds are the most competitive and usually the first group that emerges and grows simultaneously with the rice crop for a considerable time period (7-70 days). Weeds that emerge before or simultaneously with rice crop are far more competitive than those that emerge 2 to 3 weeks later. Sedges and broadleaf weeds emerge subsequently at the later stages of crop growth. While, under aerobic conditions, several flushes of weeds come up because the weed seeds with differential dormancies germinate as and when conditions are favorable. Initial slow-growth phase of the rice crop is critical when the weed growth is fast. The weeds should be prevented at this particular stage until the crop enters the fastgrowth phase, the influence of weed competition can be greatly reduced. In case of direct-sown rice, the initial 7-35 days is considered to be the most critical for cropweed competition. In uplands, since short duration varieties are grown, the proportion of their life cycle infested by weeds is higher and hence the crop suffers more (Saha and Rao 2011). Direct-sown rice in rainfed lowlands encounters similar situation as that of uplands during the initial stage and experiences competition from mainly grassy weeds and few sedges. However, with the accumulation of rainwater in crop field during peak monsoon in lowlands, the crop faces competition from some nongrassy broadleaf and aquatic weeds. In transplanted rice, weed problems are generally of lower magnitude provided the puddling and water management are done properly. Majority of weeds (about 60%) emerge within 7-30 days after transplanting (DAT) and compete with rice plants till maximum tillering stage. About 15-20% of the weed populations emerge in the period between 30-60 DAT and 20-25% of weeds emerges later and are not important in yield reduction. While sedges and broad leaf weeds are mostly predominant in irrigated ecology under both wet seeded and transplanted cultures. In wet seeded system, the crop-weed competition is more intense (because of similarities in age of rice and weed seedlings) than that of transplanted system (where aged seedling with better competitive ability are raised). The ultimate loss in grain yield due to competition with weeds is more in direct than transplanted rice irrespective of growing season.



## 2.2. Weed management under changing climatic scenario

It is expected that growth of  $C_3$  plants would be enhanced more by  $CO_2$  enrichment as compared to  $C_4$  plants. Due to greater adaptability, weeds will achieve a greater competitive fitness against the crop plants with a changed climate (Table 1). Probable changes in the weed biogeography of agricultural systems pose challenges to management. Environments with high degree of disturbance are more susceptible to annexation by newly introduced plant species and are likely to reach a relatively quick stability with emergent climatic factors. It is predicted that climate change can reduce the effectiveness of current weed management practices. Agronomic practices for particular crops are likely to change with time and space. New classes of herbicides, cultivars, tillage system, irrigation techniques and seed sowing practices will influence the geographic distribution of weeds and their invasiveness.

Under changed climatic scenario, temperature, precipitation, wind and relative humidity may influence the efficacy of herbicides. Thicker cuticle development or increased leaf pubescence, with subsequent reductions in herbicide entry into the leaf is expected in drought situation. These physiological changes can interfere with crop growth (reduced transpiration) and recovery after herbicide application. Overall, herbicides are most effective when applied to weeds those are free from environmental stress. For example, rising atmospheric CO<sub>2</sub> concentrations can reduce the glyphosate efficacy. High concentrations of starch in leaves in C<sub>3</sub> plants grown under high CO<sub>2</sub> environment might interfere with herbicide efficacy. Elevated temperature and higher metabolic activity in C<sub>3</sub> weeds tend to increase uptake, translocation and efficacy of many herbicides, while moisture deficit, especially when severely depressing growth, tends to decrease efficacy of post-emergence herbicides, which generally perform best when plants are actively growing.

Weed species	Crop	Favored underelevated CO <sub>2</sub>
Amaranthus retroflexus ( $C_4$ )	Soybean $(C_3)$	Crop
Amaranthus retroflexus ( $C_4$ )	Sorghum ( $C_4$ )	Weed
Chenopodium album $(C_3)$	Soybean $(C_3)$	Weed
Taraxacum officinale $(C_3)$	Lucern $(C_3)$	Weed
Albutilon theophrasti $(C_3)$	Sorghum ( $C_4$ )	Weed
<i>Taraxacum and Plantago</i> $(C_3)$	Grasses (C <sub>3</sub> )	Weed
Red rice $(C_3)$	Rice $(C_3)$	Weed
Echinochloa glabrescens ( $C_4$ )	Rice $(C_3)$	Weed

Table1. Crop/weed competition outcome at elevated CO,	conditions.
---	-------------

Source: Modified from Bunce and Ziska 2000

Mechanical and manual removal of weeds are the most widely used weed management practices in developing countries. Mechanical control of perennial weeds is likely to be adversely affected by elevated  $CO_2$ . Elevated  $CO_2$  could lead to increase in below ground carbon storage with subsequent increases in the growth of roots or rhizomes. This may consequently help additional plant propagation with mechanical



tillage (e.g. Canada thistle) (Ziska et al. 2004). Perennial grasses and sedges like *Cynodon dactylon, Cyperus sp.* and *Schoenoplectus articulatus* propagate asexually, hence, disking/harrowing would result in greater number of propagules. Increased photosynthesis may stimulate more production of rhizomes and other storage organs which will make control of perennial weeds more difficult. Biological control of weeds is likely to be affected. Elevated CO<sub>2</sub> could alter the efficacy of weed bio-control agents by possibly changing the development, morphology and reproduction of the target pest. Direct negative effects of high CO<sub>2</sub> in environment would be related to variations in C: N ratio and changes in the feeding habits and growth of natural enemies.

## 2.3. Challenges of weed management in direct seeded/ aerobic systems

Dry direct seeding of rice (DSR) with subsequent aerobic soil conditions eliminates the need for standing water, thus reducing the overall water demand and providing opportunities for water and labour savings. Dry seeding of rice is now considered to be an emerging production system in India and Asia because of a reasonable shortage of water availability in agriculture. Despite the numerous benefits, DSR systems adoption by farmers has been seriously inhibited by weed management tradeoffs. The practice of DSR has resulted in a change in the relative density of weed species in rice crops. In particular, *Echinochloa* spp., *Ischaemum rugosum, Cyperus difformis, and Fimbristylis miliacea* are widely adapted to conditions of DSR (Rao et al. 2007). Presence of *Leptochloa chinensis* and *Dactyloctenium aegyptium* is widely reported from many areas, particularly in DSR.

Weed management in DSR is considered a serious threat and the risks of yield losses is very high due to weed competition than in transplanted rice because (1) early flooding suppresses initial flushes of weeds early in transplanted rice but not so in DSR (2) rice seedlings in DSR are less competitive with concurrent emerging weeds because of the absence of a size difference between the rice and weeds in DSR (Rao et al. 2007). Although herbicides are important in reducing weed competition and helpful in ensuring adequate yields under DSR, overreliance on herbicides poses both economic and environmental risks. It can result in shifts in weed communities and evolution of herbicide-resistant weed populations (Rao et al. 2007) that reduce herbicide efficacy and increase costs, as newer and more expensive herbicides may be required as the relatively fast emergence of "weedy" rice. This weed is phenotypically similar to rice cultivars but exhibit undesirable agronomic traits, viz. shattering. It is usually observed in areas where DSR is being practised, and this is a serious concern to the rice production system sustainability.

In view of these weed management challenges in DSR, as well as the potential problems associated with the overuse of herbicides, several recent works have highlighted integrated weed management approach such as integration of use of competitive cultivars, changes in seed rate, timing and geometry, use of residue mulching, crop rotation, water and nutrient management, and mechanical methods (Matloob et al. 2015; Rao et al. 2007). These works have also outlined the importance of preventive measures which include seed predation, seed decay, and fatal germination



as important components of integrated weed management in DSR. However, detailed reviews on the potential of preventive approaches based on knowledge of the ecology of weed species is limited in DSR system.

In our previous study at ICAR-National Rice Research Institute, it was observed that grassy weeds viz. *Echinochloa colona, E. crus-galli, Leptochloa chinensis, Dactyloctenium aegyptium, Digitaria sanguinalis, Panicum repens* etc. are the most competitive weed-flora that emerge early and grow simultaneously with the rice crop for a considerable time period in direct-sown rice. Sedges viz. *Cyperus iria, C. difformis, Fimbristylis mileacea* etc. and broad leaved weeds *Alternanthera sessilis, Ageratum conyzoides, Ludwigia octovalvis, Sphenoclea zeylanica, Cleome viscosa etc.*) emerge subsequently at later stages of crop growth (Munda et al. 2017). Sometimes several flushes of weeds come up as seeds present in soil germinate as and when conditions are favourable in aerobic soil.

## 2.4. Hazard of herbicide resistance

Herbicide resistance is the inherent ability of a biotype of a weed to survive herbicide application to which the original population was susceptible. Herbicide resistance causes changes in the weed population because of resistant biotypes. Resistant biotypes are build up when the herbicide to which those individuals are resistant is used repeatedly. Herbicide-resistant weeds have been an issue since the early 1970s, although it was described as a potential problem as early as 1957 by CM Switzer. Like other organisms, random genetic mutations occur within plant populations. These mutations are often at very low frequencies. For herbicide resistance, a single plant in several million may have a mutation to survive herbicide treatment. Generally, herbicide applications do not cause any genetic mutations. Applications create selection pressure that favors the spread of resistant biotypes. Cross resistance can occur within weed populations.

The development of herbicide resistance poses three serious problems:

- i. Very expensive and time consuming to test for and develop alternative management plans.
- ii. Develop management techniques to continue utilizing current herbicides and protect them against resistance development.
- iii. Development of herbicide resistance in a biotype limits weed management options.

Factors that control development of resistant weeds are selection pressure, weed biology and genetic factors. If herbicides with long soil residual activity are applied repetitively, high selection pressure is placed for resistant biotypes of a weed. Some weeds have high genetic variability i.e. many different varieties or biotypes exist under the one species. They generally develop resistance quicker, as there already exist resistant biotypes within a population. Seed longevity is another factor that controls the development of herbicide resistance. Plant species that produce longlived seed tend to develop resistance early. This is because susceptible seeds from



the seed bank germinate over many years adding variation to the population. The site of action of the herbicide on the plant is governed by genetic factors. There are differences pertaining to the frequency of mutations occurance at different biological target sites within plants. Sites that have high frequency of mutation, tend to develop quickly, for example resistance may develop with three or more years of continuous use at the site of action of ALS and ACCase inhibitors. In contrast, the target site of glyphosate do not mutate as frequently. Glyphosate resistance did not exist earlier, but it took many years to develop (Neve et al. 2011).

In general, herbicide-resistant weeds are likely to develop in fields under conservation tillage (minimum and no-till systems) as congenial environment is created with repeated application of high dose herbicides. Because of the reduced tillage, farmers rely primarily and, sometimes, solely, on herbicides for weed control, thereby imposing constant selection pressures on weeds. However, the intensity of selection pressure depends on herbicide family and type of tillage operation. Reports suggested that an escalation in the use of ACCase-inhibitors in conservation-tillage did not escalate the development of wild oat populations resistant to ACCase-inhibitors. Also, the onset of glyphosate resistance in rigid rye-grass was delayed in a minimum-tillage system.

## 2.5. Newly emerging weeds/weedy rice

During the last one decade, it was observed that *Leptochloa chinensis* emerged as one of the dominant grassy weed species in rice-rice cropping sequence where the rice field became wet during the major part of crop growing season. The dominance of grassy weeds (>60% of total weed population) was recorded in DSR plots during both wet and dry season. *Leptochloa chinensis* and *Cyperus difformis* were dominant species occupying 56% of total weed population in this system (CRRI Annual Report 2014-15). *Alternanthera philoxeroides*, which generally occurs in irrigation channels, water courses, wetlands during dry season (Feb-March) at high temperature, now become an emerging weed in rice field, may be due to changes in atmospheric temperature. *Ludwigia adscendens*, another weed generally occurs in wet lands or irrigation channel, has now became another emerging weed in rice-rice system in many States of eastern India, particularly lowlands due to continuous wet condition of rice fields (CRRI Annual Report 2013-14).

Weedy rice is a troublesome weed in many rice growing regions. The extent and type of competition imposed on cultivated rice by weedy rice depends on the structural, biological and physiological features of weedy rice which shows a wide variability among different populations (Londo et al. 2006). By definition, weedy rice is an introgressed form of wild and cultivated rice (*Oryza sativa* L.). Weedy rice belongs to the *Oryza* genus and *sativa* species as cultivated rice but with different form. It appears as hybrid swarms due to introgression of genes between wild and cultivated species in nature. In Asian rice, it is known as *Oryza spontanea* whereas in the African context it is known as *Oryza stapfii*. The most common feature among extremely variable weedy rice is their ability to disseminate seeds by early shattering. It is more problematic in the direct-seeded rice than transplanted rice. The potential ecological



risks associated with transgene escape through gene flow (or crosspollination) are the foremost concerns. The spread of weedy rice infestations have been reported to 40-75% of the total rice area in Europe, 40% in Brazil, 55% in Senegal, 60% in Costa Rica and 80% in Cuba. In Asia, infestation of weedy rice became an emerging problem since 1980s. Its infestation was first reported in Malaysia in 1988, in the Philippines in 1990, and in Vietnam in 1994. Weedy rice infestation in Asia caused yield losses ranging from 16 to 74%. Yield loss of about 1 t ha<sup>-1</sup> was caused by infestation of 35 weedy rice panicles m<sup>-2</sup> as reported in Malaysia. It was reported from USA that the yield of cultivar New-bonnet was reduced by 219 kg ha<sup>-1</sup> with one weedy red rice plant in square meter. The competitive ability of one weedy rice plant was equivalent to three rice plants (cultivar Mars).

Under a competitive environment, weedy rice competes well and utilizes resources more efficiently than the cultivated rice varieties. Therefore, study of nutrient (NPK) removal by weedy rice is essential to estimate the actual loss of nutrients from soil. In Asia, some weedy rice accessions have been found to have greater nitrogen-use efficiency for shoot biomass than cultivated rice (Dar et al. 2013). Researchers have given due attention to the mechanism of nutrient losses in soil, particularly N, but only few studies have been made on the impact of weedy rice competition on nutrient use efficiency and other major nutrients P and K. Limited studies have been made on the extent to which weedy rice populations, particularly of Indian subcontinent, can compete with cultivated rice for the three major nutrients (NPK).

At ICAR-NRRI, five thousand thirteen germplasm including 41 wild rice accessions collected from Assam and 139 wild and weedy rice accessions collected from Odisha during 2012 were sown and transplanted along with check Swarna for characterization and seed multiplication. One hundred and eighty wild and weedy rice were characterized based on agro-morphological traits as per the descriptors. Study on their genetic variation suggested that the genotypes selected for this study harbored enough genetic divergence. However, an UPGMA dendrogram based on the genetic relationships suggested a closer relationship of weedy and wild rice occurring within the same regions (CRRI Annual Report 2013-14).

# 3. KNOWLEDGE GAPS

- Robust herbicide management technologies are not available to address the issues
  of herbicide persistence in soil (causing environmental pollution) and herbicide
  resistance in weeds. Greater strides need to be made to make herbicide use safer
  for environment.
- Very little progress is made in the area of weed competitive rice varieties. Weed competitive rice varieties could be a cost effective measure for suppressing the weeds.
- Further research is needed regarding the development herbicide tolerant rice. There is need to clearly understand the tradeoffs of herbicide tolerant rice.





# 4. RESEARCH AND DEVELOPMENT NEEDS

Research and development needs have been broadly discussed under the following heads:

### 4.1. Rational use of herbicides

In an agricultural system, the aim is to produce the highest yield achievable whilst minimizing costs. Herbicides are one of the first labour and costs saving technologies. Improved weed control with herbicides has the potential to improve crop yields. Chemical weed control can provide a pro-poor technology for rice cultivation in Asia. The herbicide use in the tropical countries is directly related to the cost and availability of labour. The use of herbicides has gained importance due to rise in farm wages in the recent years as a consequence of overall economic growth and growth in non-farm employment opportunities, predominantly in Asia. Recent government mandates such as the National Rural Employment Guarantee Act has created agricultural labor shortages in India because guaranteed employment and wages mandated by the Act, making hand weeding an unsuitable practice (Toth 2011).

While the role of herbicides in improving crop productivity has long been recognized, the abuse of these chemicals has been among the major causes of environmental pollution. Increasing concern has triggered research on the fate and effect of continuous and massive use of pesticides to the environment. Smallholder farmers face a number of problems associated with herbicide use, due to either an inadequate knowledge about rate of application, or the optimum time for herbicides application to control the weeds. A major cause of this is possibly serious lack of information available to the farmers and the poor level of understanding. Often only nominal precautions are taken for safe use of herbicides. Frequent herbicide application has led to herbicide resistance within some weed populations. In the USA, Propanil application for 30 years continuously, resulted in resistant Echinochloa sp. Continuous use of Bensulfuron for four years resulted in resistance in four aquatic weed species. The evolution of herbicide resistant weeds is a real threat to effective weed control where herbicides are frequently used. Smallholder systems may be particularly vulnerable as herbicides are often not used at appropriate times or dosages, which may hasten the development of resistance. To prevent and manage existing herbicide resistant biotypes requires an integrative approach. This may include research on crop and herbicide rotation, standardization of herbicide mixtures (including tank mix) and development of herbicide with short residual activity and intensive use of farm machinery to reduce herbicide load.

Persistence of any pesticide is critical for weed control. Shorter than expected activity (less persistent herbicide) can lead to poor weed control and require additional action and expense by the farmer to supplement weed management. Residual activity longer than expected (more persistent herbicide) can lead to problems with injury to a subsequently crop and may cause non point pollution (Fig. 1). The persistence depends on the characteristics of the pesticide itself or its metabolites. Volatility, solubility, formulating agents, the method and site of application of pesticides



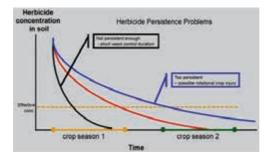


Fig.1. Herbicide persistence in soil. Source: http://ucanr.edu/blogs/blogcore/ postdetail.cfm?postnum=5929

determine the persistence. Among the environmental factors. particularly temperature, moisture and wind determine the dissipation of pesticide. Soil characteristics like organic matter content, soil pH, soil structure and texture, nutrient content and its microbial population control the persistence of any pesticide. So, further research in required in this regard to developed herbicide which are safer to use and which allow much greater plasticity in application.

In rice, a number of herbicides like butachlor, pretilachlor, pendimethalin, oxadiazon, anilofos, oxadiargyl etc. have been recommended as pre-emergence control of early flashes of weeds. Pre-emergent herbicides are usually useful in direct-sown rice fields to suppress the early flashes of weeds. These herbicides generally have narrow spectrum of controlling annual grasses and some sedges. Their efficacy depends on soil moisture and is ineffective in dry soil conditions. However, on light soils, heavy rains may move the herbicide down in the soil to the germinating crop seeds and cause severe injury. These herbicides also show severe phyto-toxic effects to rice crop emergence under flooded condition immediately after herbicide application. Mild phyto-toxicity may cause extension of flowering time and total duration of rice crop. The high application rates of pre-emergent herbicides also show detrimental effect to the beneficial microorganisms extant in soil.

In recent times, some new post-emergent herbicides with low dosages viz., bispyribac sodium, cyhalofop butyl, fenoxaprop-p ethyl, ethoxysulfuron, penoxulam, azimsulfuron, flucetosulfuron etc. and herbicide mixtures like azimsulfuron + bispyribac sodium, fenoxaprop-p ethyl + ethoxysulfuron, bensulfuron methyl + pretilachlor, cyhalofop butyl + penoxulam, metsulfuron methyl + chlormuron ethyl etc. are showing promise for controlling weeds in rice fields. The rate and time of application of these new generation herbicides/ herbicide mixtures were standardized to keep the weeds under control during first 5-6 weeks of rice crop establishment. Thus, low-dosage high-efficacy post-emergent herbicides/ herbicide mixtures having broad spectrum of weed control are expected to be an intervention to suppress the weeds during critical period of crop-weed competition up-to 35-40 days of weed emergence (Munda et al. 2017; Saha et al. 2016). Among herbicide tested at ICAR-NRRI, the lowest weed biomass (9.0 g m<sup>-2</sup>) was recorded in the Azimsulfuron + Bispyribac sodium treated plots with the weed control efficiency of 89% (ICAR-NRRI Annual Report 2014-15).

However, for successful control of weeds by herbicides, it is very much essential for the users to know different types of herbicides, specific herbicides to control different types of weed species, their doses and time of application, and safe handling and accurate application technologies for effective and environmentally safe weed control. Correct use of herbicides is essential to ensure that chemical residues on



crops do not exceed the limits. Recommended herbicides generally do not pose any threat to people, livestock, or rice crops if used correctly and if suggested precautions are followed. However, the herbicides are potentially hazardous if not handled properly.

### 4.2. Weed competitiveness

Although, herbicides provide opportunity for relatively cheap control of weeds, relieving farmers of a heavy financial burden, the over-reliance on chemicals has also led to a number of environmental and agronomic concerns. The application of herbicides is leading to the reduction of non-weedy species and having impacts on biodiversity and ecosystem function. More notably, from a production viewpoint, herbicide resistance is now a common phenomenon and widespread amongst many problematic weed species in many countries, encouraged by the increasing dependence only on a few selected herbicides. In response to these challenges, there is new interest in the prospective for integrating non-chemical (or 'cultural') control options into weed control strategies. Competitive rice cultivars would offer a relatively cheap option in integrated weed management strategies. Many cultural methods can be integrated but, competitive cultivars are a potentially attractive option in comparison, because they do not incur any added costs. Breeding weed-competitive cultivars requires easily used selection protocol, based on traits that can be measured under weed-free conditions. Such cultivars may be more capable of reducing the competitiveness of a weed species, produce chemical exudates (allelochemicals) thereby reducing the economic burden by resisting weed growth and yield loss. Competitive cultivars could reduce the seed return of a weed species and contribute to medium to long-term weed management strategies, reducing the pressure on herbicides and improving the sustainability of cropping systems.

Variability in cereal cultivars in their ability to restrict yield losses from weed competition has been demonstrated in different crops. However, such comparative studies are of limited value outside of the experimental pool of cultivars. It is important that more practical approaches are developed that can be used to assess new cultivars for various situations and guide crop breeding efforts in future. Two aspects of cultivar competitiveness can be defined. The first is the ability of the crop to reduce the fitness of a competitor, and the second is the ability of the crop to withstand the competitive impact of neighbors (suppressive ability) and resist yield loss (tolerance ability). A strong suppressive cultivar can reduce seed production capacity of weeds, which could a viable long-term strategy in weed control. By contrast, tolerance means yield will be maintained under weed pressure. Although cultivars with high competitive ability have been recognized in many cereal crops (including wheat and barley), competitiveness has not traditionally been considered a priority by rice breeders.

At NRRI, above hundred early maturing rice germplasms (95-115 days duration) with five checks viz., Vandana, Anjali, Heera, Annada and Kalinga III were screened for weed competitiveness during kharif 2014. The germplasms viz., IC 426096, RH 145-55, Jhum Fulbadam, Deng-deng, IC 337590, IC 298485, IC 447256, CR 453 and DBT 2722 were found to be weed competitive (ICAR-NRRI Annual Report 2014-15). The germplasms viz., IR 83929-B-B-291-2-1-2, IR 83750-B-B-145-4-174-3, IR-84899-B-184-



18-1-1, IR-84887-B-153-33-1-1-3, IR-84887-B-157-38-1-1-3, IR 83750-B-B-145-4-174-2 and IR 82589-B-B-63-2-148-1 were found to be weed competitive at a different location, Santhapur (CRRI Annual Report 2013-14).

A number of relationships between competitive ability of crop and plant traits have been reported in the literature, viz. plant height, early vigour, tillering, canopy architecture, belowground traits, and nutrient partitioning. These traits are not independent of one another and have implications for other plant functions in addition to weed competition, including yield potential and tolerance of stress. To realize the potential of competitive crop cultivars, a faster, cheaper and simple-to-use protocol for measuring the competitive potential of new cultivars is essential; it is likely that this will not be based on a single trait, but will need to capture the combined effect of multiple traits. Further work would be required to measure the trade-offs and recognize win-win traits that improve competitive ability without negotiating other plant functions.

### 4.3. Herbicide tolerant (HT) rice

Herbicide tolerant (HT) rice may offer rice farmers a vital tool to control broadspectrum of weeds. Rice varieties with an herbicide resistant gene would allow farmers to use an herbicide that is more environmentally-friendly than those in current use while simultaneously allowing better management of weeds. With HT-rice, farmers get the flexibility to apply herbicides only when needed. Farmers can make decision on input of herbicides with preferred environmental characteristics. HT-rice can control the weed flora associated with rice, especially of wild and other weedy rice species and also provide an alternative tool for the management of weeds that have already evolved resistance herbicides, especially grassy weeds like *Echinochloa spp*. It furthermore allows for the substitution of some of the currently used herbicides by other non-selective herbicides having less detrimental effect to the environment like glyphosate, glufosinate etc. Compared to other conventional herbicides used in rice, imidazolinone, glyphosate and glufosinate are considered environmentally benign.

There is a negligible threat of residual effects of glyphosate in soil as it is strongly adsorbed to the soil and crops. These herbicides can be used as post-emergent and therefore their rates can be adjusted to the actual weed pressure. Compared to conventional herbicides, HR-rice also provides a broader window herbicide application in terms of time frame and therefore alleviates some of the usual concerns (time pressure) for rice farmers. However, for season-long and broad-spectrum weed control, appropriate herbicide programs need to be developed for HR-rice in relation to the time of application, dose, herbicide mixture, and integration of non-chemical methods to ensure the long-term benefits of HR-rice technology.

### 4.4. Exploring herbicides – microbe compatibility

In modern agricultural production, herbicide application is one of the inevitable practices is being followed to minimize weeds problems in crop production. The indiscriminate usage of herbicides is reported to affect a group of organisms such as bacteria, fungi, nematodes, earthworms, termites and protozoa. The interaction between herbicides and soil biota is gaining practical significance since some of the herbicide molecules have adverse effects to microbial activities in soil. Many scientific evidences have revealed that the herbicides can cause both qualitative and quantitative change in soil enzyme activity. However, positive relationship may still exist with some herbicides molecules as noted in some studies. Herbicides application has both positive as well as negative effect to microbial activities in soil in response to different herbicides application.

Earlier, butachlor application was found to increase the reproductive ability of bacteria, but it is affecting the multiplication of free living nitrogen fixing bacteria particularly of Azotobacter sp. Some research findings indicated that application of organophosphates herbicides gradually increased azotobacter, arthrobacter, heterotrophic aerobic bacteria, actinomycetes and fungal counts. It is reported that herbicide viz., MCPB, bentazon, MCPB + fluozifop-p-butyl., bentazon+fluozifop-pbutyl, metribuzin, flouzifop-pbutyl+metribuzin, cycloxydin, and sethoxydin significantly increased the population of soil fungi (4 to 10 times higher) as compared uninoculated control, but these herbicides did not have any significant effect on nitrogen fixing bacteria. Different herbicides viz., pendimethalin, oxyflourfen, pursuit and pertialachlor application found gradually increased bacteria, fungi, actinomycetes and rhizobia. Arbuscular mycorrhizal fungi (AMF) is beneficial symbiotic endophytic fungi form a bridge between plants and soil and play a major role in the flow of energy and mobilization of nutrients (particularly P) from soil to plants. Other beneficial effects viz. plant growth promotion, inducing stress tolerance and enhancement of crop yield have been established. The field application of diuron and trifiuralin herbicides at recommended rates recorded minimal effects on AM fungi association. In another study, triffuralin and diuron had little adverse effect on AMF formation. As the soil has mixed population of AM fungi, understating the side effects of herbicides on these non-target microorganisms are very complex. Because species of AM fungi differ in their response to a particular chemical, so no generalization can be made on the toxicity of a chemical to AM fungi.

In rice cultivation, herbicide application is essential but there effects on nontargeted organisms need to be studied in-depth. Moreover, information on proper herbicide use is very important for preserving beneficial microbes in soil. Hence, it is essential to study the effect of new herbicides on microbial properties in rice soils. More research is needed to better understand the different aspects of microbeherbicide interactions. If we carry out some systematic studies on herbicides usage and their influence on soil microbial properties in rice based cropping systems, it will help to identify safe herbicides for rice cultivation, which in turn will preserve the soil beneficial microbes.

One of the most popular rice herbicide, Bispyribac sodium was evaluated in rice cv. Naveen at NRRI during 2015 under glass house condition to study its effect on AM fungal association. The results indicated that application of Bispyribac sodium even at double dose (600 ml ha<sup>-1</sup>) did not show any inhibitory effect to AM fungal root colonization and sporulation in rice, the same treatment which recorded 33.3 and



9.09 % higher AM fungal colonization and sporulation respectively as compared to recommended dose (300 ml ha<sup>-1</sup>). Similarly the AM fungi treated rice plants recorded significantly higher soil microbial biomass carbon (261.2 - 266.6  $\mu$ g g<sup>-1</sup> soil) after 30 days application of bispyribac sodium as compared uninoculated control (results unpublished).

## 5. WAY FORWARD

Wide variation in geographic, socio-economic, and agro-climatic conditions in rice-growing areas have resulted in equally diverse and contrasting extremes of weed control methods ranging from purely manual in developing countries to high-energy input technology in developed countries with large commercial farms. However, no single weed management strategy will solve all weed problems in rice. New management strategies will be needed as the established methods may no longer work in the changing environment. Current weed management technology consists of integration of appropriate crop husbandry (agronomic practices) along with direct cultural and chemical methods. It is also essential to develop weed database including noxious weeds in different ecosystems to track the weed dynamics. Such information will help to prepare list of weeds which are potential invaders and different models can be made use for prediction of invasiveness. The overall objective of proper crop management is to reduce the degree of direct control inputs. Thus, an integrated approach involving appropriate crop husbandry (indirect weed management strategy) including cultivation of weed competitive varieties and/or herbicide tolerant rice, good land preparation, proper water and fertilizer management, appropriate seeding rate/plant spacing, crop rotation is likely to improve rice grain yields. Future research on direct weed management practices viz. selection of herbicides with robust herbicide management strategy along with mechanical methods would bring about substantial dividends.

#### References

- Chauhan BS, G Mahajan, V Sardana, J Timsina and Jat ML (2012) Productivity and sustainability of the rice-wheat cropping system in the Indo-Gangetic Plains of the Indian subcontinent problems, opportunities, and strategies. Advances in Agronomy 117:315-369.
- CRRI Annual Report (2013-14) Characterization of the germplasm for agro morphological traits and molecular aspects. Exploration, characterization and conservation of rice genetic resources, pp. 17-18
- CRRI Annual Report (2013-14) Field performance of single row power weeder. Enhancing Productivity, Sustainability and Resilience of Rice Based Production System, pp. 68-69.
- CRRI Annual Report (2013-14) Screening of germplasm against weed Competitiveness. Enhancing Productivity, Sustainability and Resilience of Rice Based Production System, p. 73.
- ICAR- NRRI Annual Report (2014-15) Screening of early maturing germplasms for their weed competitiveness. Enhancing Productivity, Sustainability and Resilience of Rice Based Production System, p. 91.
- ICAR- NRRI Annual Report (2014-15) Study on weed spectrum and efficacy of lowdose herbicide/ herbicide mixtures under varying seed rate in dry-direct sown rice. Enhancing Productivity, Sustainability and Resilience of Rice Based Production System, p. 91-92.



- Kumar V and Ladha JK (2011) Direct seeding of rice: recent developments and future research needs. Advances in Agronomy 111:297–413.
- Londo J P, Chiang Y C, Hung K H, Chiang T Y and Schaal BA (2006) Phylogeography of Asian wild rice, Oryza rufipogon, reveals multiple independent domestications of cultivated rice Oryza sativa. Proceedings of National Academy of Sciences USA 103:9578"9583.
- Mahajan G, Chauhan BS and Gill MS (2013) Dry-seeded rice culture in Punjab state of India lessons learned from farmers. Field Crops Research 144:89–99.
- Matloob, A, Khaliq, A and Chauhan, BS (2015) Weeds of direct-seeded rice in Asia problems and opportunities. Advances in Agronomy 130:291–336.
- Moss SR, Marshall R, Hull R and AlarconReverte R (2011) Current status of herbicideresistant weeds in the United Kingdom. Aspects of Applied Biology 106:1–10.
- Munda S, Saha S, Adak T and Jambhulkar N (2017) Weed management in aerobic rice role of establishment methods and herbicides. Experimental Agriculture Doi: 10.1017/ S0014479717000576.
- Neve P, J K Norsworthy, K L Smith, and I A Zelaya (2011) Modeling glyphosate resistance management strategies for Palmer amaranth (*Amaranthus palmeri*) in cotton. Weed Technology 25:335–343.
- Rao AN, Johnson DE, Sivaprasad B and Ladha JK, Mortimer AM (2007) Weed management in direct-seeded rice. Advances in Agronomy 93: 153–255.
- Saha S, Munda S and Adak T (2016) Efficacy of azimsulfuron against complex weed flora in transplanted rice (*Oryza sativa*) under rainfed shallow lowland. Indian Journal of Agricultural Sciences 86 (2): 186–91.
- Saha S, Patra BC, Munda S and Mohapatra T (2014) Weedy rice problems and its management. Indian Journal of Weed Sciences 46(1):14-22.
- Saha S and Rao KS (2011) Major weeds of rice. Research Bulletin No. 4, Central Rice Research Institute, Cuttack, Orissa, India, pp. 1-63.
- Singh S, Bhushan L, Ladha JK, Gupta RK, Rao AN and Sivaprasad B (2006) Weed management in dry-seeded rice (*Oryza sativa*) cultivated in the furrow-irrigated raised-bed planting system. Crop Protection 25:487-495.
- Toth SA (2011) Population prosperity: India. Farm Chemicals International. Meister Media 25(10):24-30.
- Ziska, LH and K George (2004) Rising carbon dioxide and invasive, noxious plants: Potential threats and consequences. World Resources Review 16(4):427-447.\*



# Economic and Eco-friendly Use of Rice Straw

### P Bhattacharyya, H Pathak, AK Nayak, P Panneerselvam, MJ Baig, S Munda, D Bhaduri, BS Satapathy, M Chakraborti, NT Borkar and N Basak

### SUMMARY

There are about 731 million tons of lignocellulosic rice straw generated in the world every year. Every kilogram of harvested rice is accompanied by production of about 1.0-1.5 kg of the straw. Assuming that 50% of crop residues are utilized as cattle feed and fuel, the nutrient potential of the remaining residue is 6.5 million tonnes of NPK per annum. In India about 16% of generated crop residues are burnt on farms. Out of this 60% is paddy straw. Recent estimate showed that during November-December, around 70% cause of air pollution in New Delhi and its surrounding cities was straw burning. Not only Punjab and Haryana, straw burning is spreading over other states, very rapidly. Primarily burning causes emission of  $CO_2$ , CO, SOx, NOx, particulate matter and  $CH_4$  which increases air pollution and GHGs/ Carbon footprint tremendously.

It is a paradox that on one hand we have a shortage of animal feed, biofuel and manures, and on the other hand considerable amount of crop residues are either wasted or burnt. This is not only a big loss of natural renewable resources but at the same time it is a source of greenhouse gas (GHG) emissions and environmental pollution. However, these residues can effectively be used as mulch, for production of manure, ethanol, bio-diesel, biochar, etc., and in conservation agriculture. In a rough estimate, if 20% of world's rice straw is used for production of ethanol annually, about 40 billion litres could be generated, which is able to replace 25 billion litres of fossil fuel based gasoline.

There are knowledge gaps on the economic technologies for *in-situ* and *ex-situ* composting of straw, characterization of rice straw of available varieties for fodder quality, cost effective small scale technologies for bio-energy production, technologies for value addition of paddy straw in view of present day mechanized agriculture and authentic database on contribution of straw burning in air pollution and GHGs/ carbon footprint.

However, a huge potential to use paddy-straw in bio-energy production, composting, as fodder and so on, can establish it as an important bio-resource. Microbial composting, is an up-coming technology for agricultural wastes disposal in which biodegradation of lignocellullosic matter like paddy straw is carried out exploiting ligninolytic and cellulolytic microorganisms. Breeding of new varieties of rice which not only provides good quality grains for human consumption but also superior quality straw for feeding ruminant animals and producing biofuels with increased efficiency is another innovative approach. At the same time there is an



urgent need for popularizing the array of available technology such as use for power/ electricity generation, ethanol production, biochar production, composting and raw material for paper mill/board making industry.

## **1. INTRODUCTION**

Rice straw is one of the most abundant lignocellulosic materials being produced in the world (Total, 731 million tons; Africa, 20.9 million tons; Asia, 667.6 million tons; Europe, 3.9 million tons; and America, 37.2 million tons) (Sarkar and Aikat 2013). Every kilogram of harvested rice is accompanied by production of about 1.0-1.5 kg of the straw. The major agro-residues in terms of volumes generated in India (in million metric tons–MMT) are rice straw (112), rice husk (22.4), wheat straw (109.9), sugarcane tops (97.8) and bagasse (101.3), which cumulatively amounts to approximately 620 million tons (Pandey et al. 2009). On an average these residues contain about 0.5% N, 0.2% P<sub>2</sub>O<sub>5</sub> and 1.5% K<sub>2</sub>O. Assuming that 50% of crop residues are utilized as cattle feed and fuel, the nutrient potential of the remaining residue is 6.5 million tonnes of NPK per annum.

In India about 620 million tonnes of crop residue is generated every year. About 16% of them are burnt on farms. Out of this 60%, paddy straw and wheat straw accounted for 22%. Recent estimate showed that during November-December, around 70% cause of air pollution in New Delhi and its surrounding cities was straw burning. Not only Punjab and Haryana, straw burning is spreading over other states, very rapidly. Primarily burning causes emission of  $CO_2$ , CO, SOx, NOx, particulate matter and  $CH_4$  which increases air pollution and GHGs/ Carbon footprint tremendously.

## 2. RICE STRAW BURNING

The rice and wheat system (RWS) is one of the widely practiced cropping systems in India. This cropping system is dominant in India where almost 90-95% of paddy area in Punjab, Haryana and Western UP is under intensive Rice-Wheat-System (RWS) (Ladha 2000). Widespread adoption of green revolution technologies and high yielding varieties increased both crop yield as well as crop residue. In the RWS, a short period of time is available between rice harvesting and wheat plantation and any delay in planting adversely affects the wheat crop. This coupled with use of combined harvester compels the farmers to burn the residue to get rid of it.

After harvesting, open burning of rice residues (both straw and husks) is a standard practice in Asia. Open burning of rice straw residues has harmful environmental effects. It causes greenhouse gas emissions (GHGE), including 0.7–4.1 g of  $CH_4$  and 0.019–0.057 g of N<sub>2</sub>O per kg of dry rice straw, and emission of other gaseous pollutants such as  $CO_2$ ,  $SO_2$ , NOx, HCl and, to some extent, volatile organic compounds (VOC) and carcinogenic polycyclic aromatic hydrocarbons (PAH), dioxins and furans (Oanh et al. 2011). It also affects the radiation budget of the earth. Intensive burning contributes to the formation of Atmospheric Brown Cloud (ABC) that affects the air quality and visibility (Kanokkanjana et al. 2011). Rice straw burning is also an important



source of aerosol particles such as coarse dust particles  $(PM_{10})$  and fine particles  $(PM_{2.5})$  (Chang et al. 2013), affecting regional air quality and reducing visibility as a result of the gas and particle emissions. In November 2016, in an event known as the "*Great smog of Delhi*", air pollution spiked far beyond acceptable levels. Levels of fine particle and sand-coarse dust particles hit 999 micrograms per cubic meter, where as the safety limits for those pollutants are 60 and 100 micrograms, respectively, and this event is chiefly attributed to the practice of open burning of rice straw in the neighboring states of Punjab and Haryana.

Open burning of rice residues also results in loss of major nutrients. About 40% nitrogen (N), 30 to 35 % of potassium (K) and 40 to 50 % of sulphur (S) are lost. This is a critical and widespread issue in India, Bangladesh, and Nepal which is causing depletion of soil K and Si reserves at many places (Dobermann and Fairhurst 2002). Besides, the heating of the soil, kills the useful microorganisms of the soil causing soil degradation including nutrient loss, depletion of soil organic matter (SOM), and reduction in the presence of beneficial soil biota. Incorporation of rice straw into soil without proper decomposition is creating another problem of decrease in production efficiency and an increase in greenhouse gas emissions.

It is estimated that 22,289 Gg of paddy straw surplus is produced in India each year out of which 13,915 Gg is estimated to be burnt in the field. Punjab and Haryana alone contribute 48% of the total open field burning (Gadde et al. 2009). One year of crop residue in Punjab contains about 6 million tonnes of carbon that on burning could produce about 22 million ton of  $CO_2$  in just 15-20 days, says a study published in Springer Briefs in Environmental Science (2014). The study also showed that CO levels become critical in the area surrounding a burning field: concentrations of 114.5 mg m<sup>-3</sup> or more were observed at 30 m from burning fields and 20.6 mgm<sup>-3</sup>at 150 m away. The permissible limit of CO in ambient air is 4.0 mg m<sup>-3</sup>. Significant amounts (40–50  $\mu$ gm<sup>-3</sup>) of nitrogen oxide (NO<sub>2</sub>) and ammonia (NH<sub>3</sub>) were also recorded during burning.

Open burning in the field affects life of human, animals, birds and other insects below and above the earth. Burning at times also causes poor visibility and increases the incidents of road accidents. Apart from humans and animals, residue burning also adversely impacts the soil health. According to a presentation made by GV Ramanjeneyulu, agriculture scientist and executive director of Hyderabad-based non-profit Centre for Sustainable Agriculture, before the Punjab government, heat from burning straw penetrates 1 cm into the soil, elevating the temperature to as high as 33.8-42.2°C. This kills the bacterial and fungal populations critical for a fertile soil. The presentation also showed that the monetary cost of burning to Punjab farmers is around Rs. 800-2,000 crore every year in terms of nutritional loss and Rs. 500-1,500 crore in the form of government subsidies on nitrogen, phosphorus and potash fertilizers.

Recent environmental pollution caused by straw burning open up a challenge to agricultural scientist as to how the crop residue may be better managed. What other alternative economic avenues may be exploited that will enable environment friendly usages of straw/crop residues. Integrated research approach should be addressed



to use straw/crop residues in all possible options like, biochar conversion, bio-ethanol production, better feedstock preparation for animals, as raw materials for paper industries etc. In this chapter we discuss the international and national status of rice straw management along with some alternative management strategies for *in-situ* utilization of paddy straw, value addition in the light of fodder quality improvement and substrate for mushroom production and few alternative avenues for utilization of rice straw towards bio-energy production and *ex-situ* composting.

# 3. TECHNOLOGIES FOR STRAW UTILIZATION

**China:** China being a leading rice producer, has ample generation of rice straw, contributing 62% of country's total crop residues. Few technologies are already available for utilizing the straw in the forms of fuel, feedstuff, manure and industrial raw material. For a part of the country rice straw plays as the major energy source. Since 1981, China had started a drive for better crop biomass resource and energy conversion technology, encompassing rice straw which plays an important role (Liu et al. 2011).

**Pakistan:** Farmers in Punjab, Pakistan adopt three main residue management practices, including: i) the burning of rice residue after the rice harvest or 'full burn', including the top part (pural) and the lower parts; ii) the removal of rice straw; and iii) the partial or complete incorporation of rice residue into the soil using farm machinery (rotavators and disc harrows). Though a negligible percentage of farmers followed the incorporation of rice residue into the soil, and if assessed by area, the 'full burn' method ranks first. Around 58% of the area under rice cultivation was fully burned, while the full removal of rice residue covered only 25% of the rice area in Pakistan. The remaining area was either partially burnt or had rice residue incorporated into the field.

**Sri Lanka:** In contrast, in Sri Lanka, straw is used for a number of purposes. Rice straw is widely used as fodder, for the manufacturing of paper and paper boards and for thatching the roofs of houses. Its use as mulches is also practiced in cultivation of ginger and turmeric crops. A small amount of the straw is also used as a packaging material. Only a handful of farmers add the straw back to the fields whereas a large number burn the straw at the threshing site. Majority of farmers are unaware of the value of rice straw as a fertilizer material and a sizable quantity of rice straw is wasted in Sri Lanka primarily owing to this unawareness.

**Bangladesh:** Bangladesh produces around 43.85 million MT of rice residue (EPA, 2011). For residue management in Bangladesh, several practices are followed: (a) burning in the field, (b) incorporating in the field and (c) removing from the field either for burning along with cow dung or for feeding cattle. Report says complete (100%) field burning of the residue is observed only in 3% of surveyed area, prevalent in Narail, Khulna and Faridpur districts (Haider 2011). The farmers also remove or do not burn residue instead keeping it for selling purpose. The main reason behind burning the lower part of residue in the field is to use it as fertilizer thereby eliminating expensive



operations like removal and cleaning of the land. Many farmers think that residue burning in the field provides fertilizer to the field for the successive seasons. However, higher removal cost is the main reason behind not removing the residue from the field.

**Vietnam:** In Vietnam, to chalk out the alternative rice straw management for future, a study reported that deploying rice straw for biochar production and soil amendment led to a lower climate change impact showing negative carbon footprint than open burning of biomass in both spring and summer seasons' rice cultivation (Mohammadi et al. 2016). The Mekong Delta region has appeared to be potential hotspot for bioethanol production from rice straw, producing 26 Mt rice straw yearly with an estimated cost of 1.19 \$  $L^{-1}$ . However, to lower down the production cost in terms of energy cost, Diep et al. (2015) suggested few modifications in bioethanol plants, like using residues for power generation and improving solid concentration of material in the hydrothermal pre-treatment step.

**Thailand:** In order to discourage open burning of rice straw and to promote the possibility of rice straw utilization for industrial applications, researchers of Thailand proposed the use of rice straw based stoker boiler, a promising technology for heat and power generation and an alternative to coal based power, with less emission of NOx and chlorinated organic compounds (Suramaythangkoor and Gheewala 2010).

**Japan:** The Japanese cabinet launched "*Biomass Nippon Strategy*" for efficient biomass utilization in Japan, though the amount of biomass resource is not very large and limited production scale (Matsumura and Yokoyama 2005). In that scenario, power generation from rice straw biomass (with a production potential of 12 Mt-dry year<sup>"1</sup>) had a potential to supply 3.8 billion (kW) h of electricity per year, sharing only 0.47% of the total electricity demand in Japan (Matsumura et al. 2005). In Japan, a long-term trial has been successfully conducted using crop residue management in Fukuoka, where soil quality showed beneficial impact under treatment of rice straw compost in terms of greater accumulation of total C and N in the soil from rice straw compost, with a declined value of metabolic quotient ( $qCO_2$ ) indicating better C utilization efficiency by soil microbes (Tirol-Padre et al. 2005).

**Korea:** In Korea, rice straw (lingo-cellulosic fiber) is used along with waste tire particle for manufacturing composite boards using as insulation boards, which showed good acoustical insulation, electrical insulation, anti-caustic and anti-rot properties over wood particle board (Yang et al. 2004). In another instance, regenerated cellulose fibers (with a diameter of 10 to 25 im) were prepared by wet spinning in rice straw/*N*-methylmorpholine-*N*-oxide solution (Lim et al. 2001).

**India:** India, the second largest rice producer in the world, produces nearly 130 million tons of rice straw annually. Rice straw is fed to cattle and buffaloes in India since ages. Rice straw is fed to cattle at home as basal diet in most areas where green fodder is scarce. It is also used as feed for ruminants and has many other uses like manure, thatching, purpose paper pulp, alcohol, mats, poultry litter and mushroom production. Farmers are yet to realize the importance of rice straw as a form of manure and as a profitable raw material for various industries. In the major rice growing states of



Punjab and Haryana, farmers have tried various means of disposing the rice straw while earning some income. Brick manufacturing companies, power companies, and paper and packaging industries use rice straw as a raw material.

Punjab Agriculture University recommended various new technologies for straw management. One of the promising one is to cut the straw in to small pieces and scatter over the ground mechanically after harvesting by combined harvester followed by direct sowing of the wheat with happy seeder in that field. In one day, up to 15-20 acres can be sown. The scattered straw helps not only in conserving the soil moisture but also helps to operate happy seeder for direct drilling of wheat into the rice residue in a single pass without burning the straw.

Applications of microorganisms as accelerators of biodegradation have shown promising results. A consortium of *Candida tropicalis* (Y6), *Phanerochaete chrysosporium* (VV18), *Streptomyces globisporous* (C3), *Lactobacillus* sp. and enriched photosynthetic bacterial inoculum hasten the composting process of rice straw by bringing C:N ratio down to 15:1 and achieving a total humus content of 4.82% within 60 days as reported by Sharma et al. 2014.

A combination of cow dung slurry @ 5% + *Trichoderma harazianum* @ 5 kg/ha + *Pleurotus sajorcaju* @ 5 kg/ha had significant influence in degrading rice straw as evidenced through the activity of N- fixing and P- solubilizing microorganisms in the soil. It was reported that nutrient enriched compost can be prepared by using the consortium of fungal (*Aspergillus nidulans, A.awamori, Trichoderma viride and Phanerochaete chrysosporium*) inoculants within 70-90 days by pit and windrow methods.

Some existing microbial culture available in India for composting of rice straw are listed in tabular form (Table 1).

On short-term basis, rice residue addition stimulates  $CH_4$  emission in next crop, immobilizes available N, and may accumulate toxic material in soil. In general, no consistent/significant effect of either incorporation or mulching of rice straw on N<sub>2</sub>O emission was found, however, an increase in emission of N<sub>2</sub>O from field with mulch compared to the incorporation was observed in subtropical rice-based cropping system. Bhattacharyya et al. (2012) reported that the application of inorganic fertilizers in combination with rice straw in tropical rice resulted in C build up, increase in productivity and sequestration capacity of soil, although, it also resulted in higher GHGs emissions. They recommended combination of inorganic fertilizer (urea) with rice straw (1:1 N basis) for building of soil C (1.39 Mg ha<sup>-1</sup>), sustaining crop yield and lower GHGs emission as compared to addition of rice straw/green manure alone.

# 4. ALTERNATIVE USE OF RICE STRAW

### 4.1. Conservation agriculture (CA)

Zero and/or reduced tillage is an important component of CA. It helps in increasing soil organic matter by leaving the previous crop residues on the soil surface to decay, which leads to increased soil nitrogen while conserving soil moisture and structure.



	<b>D</b>	
Type of microbes used	Recommended dosage	Source
Consortium of decomposing microbes	2.0 kg inoculum per tonne of wastes	TNAU, Coimbatore
photosynthetic bacteria, <i>Lactobacillus</i> , <i>Streptomyces</i> , actinomycetes, yeast	EM solution accelerators @33.3 lit per pit (6'x4'x3' LBH) <i>i.e</i> to produce 0.9 t compost	Indian Institute of Soil Science, Bhopal
<i>Trichoderma harzianum</i> (compost activators should be prepared by mixing <i>T.harzianum</i> with carbonaceous plus nitrogenous materials)	1.0 kg per 100 kg substrate	Indian Institute of Soil Science, Bhopal
Pleurotus sajar-caju	1.25 kg inoculum per tonne of waste (1:1.25 ratio of paddy straw and poultry dropping)	Indian Institute of Soil Science, Bhopal
Fungal consortium (Aspergillus niger, A. flavus and Trichoderma harazianum)		IARI, Delhi
Phanerochaete chrysosporium VV18, Streptomyces sp.C3, Rhodotorula glutinisY6, Lactobacillus plantarum		IARI, Delhi
Myrothecium roridum LG 7, Trametes hirsuta and Streptomyces griseorubens		IARI, Delhi
Cow dung slurry and bone meal or cow dung slurry is applied layer by layer	5-10 kg cow dung in 2.5 to 5.0 I of water and 0.5 to 1.0 kg fine bone meal sprinkled over it uniformly per layer	TNAU, Coimbatore
	Consortium of decomposing microbes photosynthetic bacteria, <i>Lactobacillus</i> , <i>Streptomyces</i> , actinomycetes, yeast <i>Trichoderma harzianum</i> (compost activators should be prepared by mixing <i>T.harzianum</i> with carbonaceous plus nitrogenous materials) <i>Pleurotus sajar-caju</i> <i>Pleurotus sajar-caju</i> Fungal consortium ( <i>Aspergillus niger</i> , <i>A. flavus</i> and <i>Trichoderma harazianum</i> ) <i>Phanerochaete</i> <i>chrysosporium</i> VV18, <i>Streptomyces sp.</i> C3, <i>Rhodotorula glutinis</i> Y6, <i>Lactobacillus plantarum</i> <i>Myrothecium roridum</i> LG 7, <i>Trametes hirsuta</i> and <i>Streptomyces</i> <i>griseorubens</i> Cow dung slurry and bone meal or cow dung slurry is applied layer	useddosageConsortium of decomposing microbes2.0 kg inoculum per tonne of wastesphotosynthetic bacteria, Lactobacillus, Streptomyces, actinomycetes, yeastEM solution accelerators @33.3 lit per pit (6'x4'x3' LBH) <i>i.e</i> to produce 0.9 t compostTrichoderma harzianum (compost activators should be prepared by mixing T.harzianum with carbonaceous plus nitrogenous materials)1.0 kg per 100 kg substratePleurotus sajar-caju A. flavus and Trichoderma harzianum1.25 kg inoculum per tonne of waste (1:1.25 ratio of paddy straw and poultry dropping)Fungal consortium (Aspergillus niger, A. flavus and Trichoderma harazianum)Streptomyces sp.C3, Rhodotorula glutinisY6, Lactobacillus plantarumMyrothecium roridum LG 7, Trametes hirsuta and Streptomyces griseorubens5-10 kg cow dung in 2.5 to 5.0 I of water and 0.5 to 1.0 kg fine bone meal or cow dung slurry is applied layer by layer

#### Table 1. Existing composting technologies of rice straw available in India

Leaving the residues on the field protect the soil surface from direct impact of wind and rain drops hence reducing wind and water erosion and conserving soil moisture. It also prevents germination of weeds. However, some disadvantages like, greater risks of crop yield reductions or failure in initial year, increased possibility of pests and diseases and difficulty in incorporating fertilizers may remain. But, with the advent of second generation farm machineries like "Happy Seeder", management of rice straw in field became easy. The Happy Seeder is a machine that cuts and lifts rice





Fig. 1. Happy seeder



Fig. 2. Straw chopper and baler (Source: www.mikeprycemachinery.com)

### 4.2. Mulch

straw, sows seeds into the bare soil, and deposits the straw over the sown area as mulch (Fig. 1).

Combine harvesters are gaining popularity day by day in India for timely harvesting of paddy. By the combine harvested fields leave high stubbles and straw in the field. For timely sowing of next crop without burning of these loose straw, chopping machine is used to pick them up and conveyed to serrated blade for chopping cylinder, and the chopped straw are then spread over the field.

Straw baling in the combine harvested paddy field is considered as technically and economically beneficial for animal feed, biofuel and other industrial uses. Straw baling technology is very useful for mechanized collection of straw and can be done in less time. It is convenient and economical for handing, transportation and storage of straw (Fig. 2) by straw bales.

Rice straw could be used as mulch for other crops like wheat, maize, sugarcane, sunflower, soybean, potato, chilli etc. In one side, it would improve crop yield in dry land and water stress condition by conserving soil moisture and on the other hand, could save irrigation water of about 7 to 40 cm. In light of reduction of GHG emission, sowing of wheat with rice straw mulching in residual moisture (without pre-sowing irrigation) could save about 20% irrigation water, which could save 80 KWh of electricity and reduce emission of 160 kg of  $CO_2$  equivalent (Singh and Sidhu 2014).

### 4.3. Biochar

Controlled pyrolysis of crop residues (CRs) could produce biochar which not only reduces GHGs emission but at the same time sequester C in soil for long time.

Biochar is a heterogeneous substance rich in aromatic carbon and minerals. It is produced by pyrolysis of sustainably obtained biomass under controlled conditions with clean technology and is used for any purpose that does not involve its rapid mineralization to  $CO_2$  and may eventually become a soil amendment (Fig. 3).

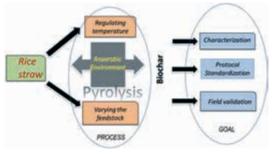


Fig. 3. Conceptual flowchart of rice straw recycling through biochar.



However, technological options and spatial field level data are required to fully utilize or recognize its GHGs mitigation/adaptation potential. Biochar serves as a sink for atmospheric CO<sub>2</sub> because of its reactive surfaces and recalcitrant aromatic structure. Further, during pyrolysis about 50% of C in rice straw is immediately released, which could be effectively used as energy sources and the remaining recalcitrant is biochar which sequester C in soil. So it is a win-win situation. On the other hand, 80-90% of C of rice straw in original form is emitted as CO<sub>2</sub> within 10 years, depending on soil and environmental constraints. Therefore, recalcitrant biochar offers a prospect of sequestering 40-50% of original rice straw-C having a huge greenhouse mitigation benefit. However, India produces only about India 1.7 million tons of biochar/year (Srinivasarao et al. 2013).

### 4.4. Rice straw as animal feed

In India, rice straw is used as fodder since time immemorial. In eastern part of the country, it is the traditionally the most popular form of dry biomass which is given to animals after they are chaffed and then mixed with green fodder or as basal diet in animal sheds. In Punjab, Haryana and western Uttar Pradesh, rice straw is used mostly as basal diet. However, the fact remain that it is the scarcity of good quality feed and fodder in the country which forces the farmers to use paddy straw as fodder. It is a poor quality feed in terms of protein and mineral content which are the two important components of a fodder crop. On an average the crude protein content in rice straw is as low as 4%. Although the crude fibre content is approximately 37% and the total ash content is nearly 18%, the major fraction of them is lignocellulose and insoluble ash. Due to these reasons, paddy straw is poorly palatable and thus its intake by animals is low.

**Rice straw-based animal feed block making machine:** It is useful for making animal feed blocks of size 20 cm x 20 cm by mixing rice straw with essential nutritional elements. The machine is powered by 25 hp electric motor and has the capacity of 250 kg h<sup>-1</sup>. It is also available in 125 kg h<sup>-1</sup> capacity with block size of 10 cm x 10 cm operated by 10 hp electric motor. The self-life of the feed blocks is more than one year, very economical to transport to distant places.

Feed block making procedure: The rice straws collected from farmers field are passed

through chaff cutter machine (15 hp capacity) and are made into pieces of about 1 to 2-inch size. The essential mineral mixtures (i.e. splitted maize grain, green gram, black gram, horse gram and jiggery) for increasing its nutritional quality and chopped rice straw are placed in the mixing machine (5 hp size) (Fig. 4). The output from the mixing machine is then passed through the feed block making unit (25 hp size)



Fig. 4. Mixing unit (Rice Straw + Other ingredients) (Source: IARI, New Delhi)



Rice Research for Enhancing Productivity, Profitability and Climate Resilience



Fig. 5. Feed block making machine (after mixing with ingredients) (Source: IARI, New Delhi)



Fig. 6. Feed block ready to use

(Fig. 5) which then makes feed blocks of 3.5 kg each (Fig. 6), which are ready for selling and are used for animal feeding (cow/buffalo).

#### 4.5. Mushroom industry

Cultivation of edible mushroom is one of the cheapest and economically viable processes for the bioconversion of lingo-cellulosic wastes. Mushrooms have capacity to convert nutritionally less valued substances like rice straw in to valuable and nutritious human food and animal feed. Various studies recommends the use of paddy straw for cultivation of different types of mushrooms viz. Button mushroom (*Agaricus bisporus*), Oyster mushrooms (*Pleurotus* spp.) and paddy-straw mushroom (*Volvariella volvacea, V.diplasia*) in India.

Mondal et al. (2010) revealed that, highest biological and economic yield of Oyster mushroom was obtained from rice straw as compared to other substrate materials tested. The highest yield from rice straw appeared, due to comparatively better availability of nitrogen, carbon and minerals from this substrate. The rice straw could be used as substrate at a concentration of 15%: 60% in planted media of Oyster mushroom production without any reduction in productivity of white Oyster mushrooms (Utami and Susilawati 2017). It is one of the best substrate for cultivation of milky white mushroom.

Paddy straw mushroom is the third most important mushroom cultivated in the world and this mushroom can use wide range of cellulosic materials and prefers C: N ratio of 40 to 60. North Eastern region and eastern India comprising of West Bengal, part of Bihar, Jharkhand and Odisha has tremendous potential and scope for paddy straw mushroom cultivation due to the easy availability of basic substrate (paddy straw). Use of rice straw for cultivation of *Volvariella* spp. will decrease the environmental problem and provide sustainable means of adding value to rice farmers (Tripathy et al. 2011).

Cultivation of *Pleurotus florida* on rice straw has beneficial effect on digestibility, degradability and increases the nutritional values for animal feed (Hadizadeh et al. 2015). Even the leftover of paddy straw after harvesting mushroom can be re-used as manure (after composting) for other crops which would save expenses on chemical



fertilizers. The proteinaceous, low lignin content of the spent rice straw from mushroom production is a potential feed quality for ruminants (Kathrina et al. 2016). Edible mushroom treated rice straw shows promise as feed resources for ruminant animals either solely or in combination with other feedstuffs.

The physical and chemical properties of rice straw varies with variety, harvest time, method of threshing and season of cultivation of rice crop which influences the quality and productivity of mushrooms. There is consistent variation in the nutrient value of rice straw associated with location and season for rice cultivars. Hand threshed rigid and tall rice straw was found to be more appropriate than dwarf cattle threshed and flexible straw against V. esculenta. Cellulose/lignin ratios in rice straw were positively correlated to mycelial growth rates and mushroom yields. The physical properties (moisture content, particle size, bulk density and porosity of rice straw varies with rice varieties even though they were grown under same climatic conditions using similar soil type and cultivation methods. Earlier findings revealed that paddy straw derived from variety CR 1014, 1242, 141, T90 is good for preparing mushroom beds. The role of extracellular enzymes like cellulases, hemicellulases and lignases is pivotal to the production of any mushroom fruiting body which is affected by the various nutrients and physical factors of the used substrates. There is a great scope to evaluate the predominant rice varieties and available rice germplasms to establish the rice varieties most suitable for economic and environment-friendly utilization of rice straw for value addition.

### 4.6. Composting

Composting is a microbiological process carried out by succession of mixed microbial populations with specific functions. High lignin content restricts the enzymatic and microbial access to the cellulose in paddy straw. Many composting technologies are available which requires nearly 60-75 days for complete decomposition of paddy straw, but there is an absence of viable rice straw decomposing methods within short period of time (45-60 days). At present, there is a dearth of viable *in-situ* decomposition of rice straw residues at field levels. Hence, it is essential to develop efficient microbial consortium to solve the aforesaid problems.

The composting of agricultural residues rich in lignocellulose like paddy straw generally takes five to six months to obtain good and mature compost. Cellulose degrading microorganisms hasten the biodegradation of crop residues such as straw, leaves, trash etc. and such cultures have been used for composting of plant residues but the time taken for composting is still too long. In nature, during decomposition of lignocellulosic material many mesophilic, thermophilic and thermo-tolerant microorganisms like fungi, bacteria and actinomycetes play a significant role at various stages. Cellulose is the main polysaccharide in terrestrial ecosystems. Rice straw has a cellulose content of 37-49%. It represents a huge source of energy for microorganisms. In nature, most cellulose is degraded aerobically with the final product being CO<sub>2</sub>. Cellulose is insoluble in water and therefore requires enzymatic degradation. The ability of bacterial and fungal communities to degrade cellulose aerobically is widespread among some soil microbial groups. Cellulose degrading bacteria are found



in both filamentous (e.g. *Streptomyces, Micromonospora*) and non-filamentous (e.g. *Bacillus, Cellulomonas, Cytophaga*) genera.

*Cytophaga* and *Sporocytophaga* are dominant cellulolytic microorganisms in most of the composting processes; these are the aerobic mesophilic bacteria able to degrade cellulose. Some mesophilic aerobic forms of *Bacillus*, like *B. subtilis*, *B. polymyxa*, *B. licheniformis*, *B. pumilus*, *B. brevis*, *B. firmus*, *B. circulans*, *B. megaterium* and *B. cereus* are also reported to behave as cellulose and hemicellulose degraders. Similarly, actinobacteria (*Streptomyces* sp) has strong biodegradative activity, secreting a range of extracellular enzymes and exhibiting the capacity to metabolize cellulose, hemicellulose and lignin (Saritha et al. 2012).

Cellulose degradation is a common trait among fungi within both Ascomycota and Basidiomycota. Aerobic cellulolytic fungi produce freely diffusible extracellular cellulase enzyme systems consisting of endoglucanases, exoglucanases and â-glucosidases that act synergistically in the conversion of cellulose to glucose (Lynd et al. 2002). Hundreds of species of fungi are able to degrade lignocellulose. There are mainly three types of fungi living on dead wood that preferentially degrade one or more wood components viz. soft rot fungi, brown rot fungi and white rot fungi. In majority of soils, 80 per cent of the fungal population belongs to the genera *Aspergillus* and *Penicillium*. However, the most extensively studied lignocellulolytic fungi are *Trichoderma, Phanerochaete* sp. and *Pleurotus sp*.

### 4.7. Biogenic silica from rice straw

Biogenic silica from rice straw has amorphous silica. It is having at least 3% of silica and preferably more than 20% by weight of silica for use as an anti-caking agent, excipient or flavor carrier. The straw is ground and the silica may be concentrated by carbon reduction through enzymatic treatment or burning. In some instances an antimicrobial treatment of the silica may be beneficial.

### 4.8. Alternate source of energy: Biofuel/biogas/bioethanol production

Rice straw being a ligro-cellulosic material is considered to be a potential source of renewable energy. In this context synthesis of biofuel from rice straw, and mixing of biofuels with convectional fuels could save the exploitation of fossil fuel thereby reducing GHGs emission while helping to mitigate climate change. According to IPCC data inventory (2014) about 25 billion tons of  $CO_2$  are generated by anthropogenic activity every year, which could be reduced by lowering emission, enhancing sink or removals and avoiding emission (through fossils fuels). All these three could be effectively done, partially by suitable/effective management of rice straw. An increasing trend has already been observed through 4<sup>th</sup> (2007) and 5<sup>th</sup> assessment (2014) report of IPCC on the use of CRs as sources of feed stock for energy to displace fossil fuel. CRs could be burnt directly or be processed further to generate liquid fuels like ethanol or bio-diesel (IPCC 2007; IPCC 2014).

There is a huge potential to offsets fossil fuel by generating ethanol from bulk CRs in general and rice straw in particular, with efficient commercial technologies.



Potentially, 250-350 litre ethanol could be produced from each metric ton of dry CRs. Considering only 20% of world's rice straw is being utilized for this purpose, lead to an annual ethanol production of 40 billion litres, which would be able to replace about 25 billion litre of fossil fuel based gasoline (Jeffery et al. 2011). As a result net GHGs emission could be reduced to a tune of 70 million tonnes  $CO_2$  equivalent per year. But large scale and small scale commercial machineries/technologies need to be developed for harnessing this potential. However, for bio-energy perspective, residue would be removed rather than returned to the soil (opposite to concept of conservation agriculture). For that harvesting, threshing and transportation mechanism need to be streamlined. Along with that, varieties having desirable straw characteristics should be grown for biofuel production. Presently only 10% of the total rice residues in India are used for bioenergy production (NAAS 2012).

### 4.9. Biogas

Biogas from rice residue in combination with animal excreta is an age old technology in rice growing tropical world. But its use and efficiency has been declining over the years, although, it has various benefits including plant nutrient addition to soil. Further, fermented/composted biogas when used as manure in rice-paddy, generate less  $CH_4$  as compared to addition of fresh organic manure. However, escalating fuel price and climate change/environmental issue may re-stimulate its future (Singh and Sidhu 2014).

# 5. KNOWLEDGE GAPS

- Lack of economic technologies for *in situ* composting.
- Absence of viable microbial inoculants for rapid *in-situ/ex-situ* decomposition of paddy straw.
- Paddy straw is relatively less preferred by animals than wheat and crop residues

   lack of characterization of available variety/germplasm for fodder quality
- Lack of cost effective small scale technologies for bio-energy production.
- Less effort has been made for value addition of paddy straw in view of present day mechanized agriculture.
- Lack of authentic database on contribution of straw burning in air pollution and GHGs/carbon footprint.
- Lack of Cataloguing/prioritizing the causes of biomass burning required for policy making to address the problem

# 6. WAY FORWARD

There is huge potential to use paddy-straw in bio-energy production, composting, as fodder and so on, which can establish it as an important bio-resource. Microbial composting, an up-coming technology for agricultural wastes disposal in which



biodegradation of lignocellullosic matter like paddy straw is carried out exploiting ligninolytic and cellulolytic microorganisms. Minimizing the time period for decomposition is considered along with the other key factors viz. C/N ratio (25-30 is optimum), temperature, aeration and moisture affecting the composting process and quality. Therefore, nutrient amendments (urea, DAP, cattle, poultry, swine manures, soybean residues, *Jatropha Curcas*) have been used for decreasing the C/N ratio of rice straw. Based upon the aerobic or anaerobic types of composting process, aeration is necessary and so is the moisture content.

Green revolution in the country was achieved through development of semidwarf versions of traditional tall *indica* varieties. This led to a reduction of lodging incidences by changing the centre of mass of the plants. However, the plant height reduction also led to reduced biomass production from a unit area. In the quest for higher yields, the selection of strong culms to sustain heavy panicles also lead to selection of genotypes with higher amount of lignocelluloses in stems. In order to develop varieties suitable for both grain production and better quality feed and biofuel, the lodging resistance can't be compromised which makes the matter more complicated. Digestibility and saccharification efficiency of the straw depends on the cell wall composition. With increased lignin content in shoots, the lodging resistance of the culm increases, whereas, the fodder quality and saccharification potential decreases substantially. Hence, an alternative mechanism where lignin content can be reduced substantially without reducing the culm strength will be a requisite.

Recently, in rice a mutant line "gold hull and internode2 (*gh2*)" have been identified as lignin deficient. The *GH2* (*OsCAD2*) gene encodes cinnamyl-alcohol dehydrogenase (CAD) enzyme. Generally the mutants have lower lignin content and are prone to lodging. A new natural mutation in the same gene was identified where the lignin content was reduced to a great extent, although high culm strength was obtained due to presence of strong, thick culms along with a thick layer of cortical fibre tissue with well-developed secondary cell walls. The new rice variety developed by this mutation produces high biomass suitable for forage and bioenergy and have been named as Leaf star (Ookawa et al. 2014). Some other QTLs have also been identified which increases lodging resistance in rice culms without increasing the lignin or silica content. Rather some QTLs like *prl5* and *lrt5* (Kashiwagi et al. 2008 and Ishimaru et al. 2008) increases the starch content in culms through carbohydrate reaccumulation in stem after grain filling.

All the above discoveries have opened new avenues for breeders for developing new varieties of rice which not only provides good quality grains for human consumption but also superior quality straw for feeding ruminants and producing biofuels with increased efficiency.

Paddy straw has economic potential but there is an urgent need to popularize the array of available technologies for straw utilization such as use for power/electricity generation, ethanol production, biochar production, composting and raw material for paper mill/board making industry.



It is also necessary to develop machinery bank at block or district level equipped with straw management machinery and help in capacity building of the farmers regarding various uses of paddy straw management through various extension strategies such as demonstration, field days and exposure visits. Development of appropriate farm machinery to facilitate collection, volume reduction, transportation and application of crop residues and sowing of the succeeding crop under a layer of residues on soil surface is also the need of the hour.

#### References

- Arora DS, Sharma RK and Chandra P (2011) Bio-delignification of wheat straw and its effect on in vitro digestibility and antioxidant properties. International Biodeterioration Biodegradation 65(2): 352-358.
- Bhattacharyya P, Roy KS, Neogi S, Adhya TK, Rao KS, Manna MC (2012) Effects of rice straw and nitrogen fertilization on greenhouse gas emissions and carbon storage in tropical flooded soil planted with rice. Soil and Tillage Research 124: 119-30.
- Binod P, Sindhu R, Singhania RR, Vikram S, Devi L, Nagalakshmi S, Kurien N, Sukumaran RK, Pandey A (2010) Bioethanol production from rice straw: an overview. Bioresource Technology 101(13): 4767-4774.
- Diep NQ, Sakanishi K, Nakagoshi N, Fujimoto S, Minowa T (2015) Potential for rice straw ethanol production in the Mekong Delta, Vietnam. Renewable Energy 74: 456-463.
- Dobermann A, Fairhurst TH (2002) Rice straw management. Better Crops International 16 (1): 7–11.
- EBC (2012) 'European Biochar Certificate Guidelines for a Sustainable Production of Biochar. European Biochar Foundation (EBC), Arbaz, Switzerland.
- EPA (2011) No. EPA 430-R-11-005, Washington, D.C., Environmental Protection Agency, 'Inventory of U.S. Greenhouse Gas Emissions and Sinks: 1990-2009', United State.
- Gadde B, Bonnet S, Menke C, Garivait S (2009) Air pollutant emissions from rice straw open field burning in India, Thailand and the Philippines. Environmental Pollution 157: 1554– 1558.
- Hadizadeh N, Kheiri F and Shakeri P (2015) Evaluation of the nutritional value and digestibility of rice straw enriched by Oyster mushroom (*Pleurotusflorida*). Cibtech Journal of Zoology 4(2): 76-82.
- IPCC (2014) In: Stocker TF, Qin D, Plattner GK, Alexander LV, Allen SK, Bindoff NL, Bréon FM, Church J.A, Cubasch U, Emori S, Forster P (2013) Technical summary. In Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press.
- IPCC [Intergovernmental Panel on Climate Change] (2007) Agriculture. In: Metz B, Davidson OR, Bosch PR (Eds.), Climate Change 2007: Mitigation. Contribution of Working Group III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom/New York, NY, USA.
- Josephine RM and Sahana B (2014) Cultivation of milky mushroom using paddy straw waste. International Journal Current Microbiology Applied Science 12(3): 404-408.
- Kanokkanjana K, Cheewaphongphan P, Garivait S (2011) Black carbon emission from paddy eld open burning in Thailand. 2<sup>nd</sup> International Conference on Environmental Science and Technology, Vol.6. IPCBEE, LACSIT Press, Singapore.



- Kashiwagi T, Togawa E, Hirotsu N and Ishimaru K (2008) Improvement of lodging resistance with QTLs for stem diameter in rice (Oryza sativa L.). Theory of Applied Genetics 117(5): 749-757.
- Kathrina MM, Evaristo AA, Daniel LA and Sofronio PK (2016) Use of effective microorganisms on enhancing the mycelial growth of *Pleurotus florida*on unsterilized rice straw. Journal of Biological Engineering Research and Review 3(1): 30-36
- Kumar S and Kumar P (2016) Economic Impact of Air Pollution from Agricultural Residue Burning on Human Health. Climate Change Challenge (3C) and Social-Economic-Ecological Interface-Building p 313.
- Ladha JK (2000) Improving the productivity and sustainability of rice-wheat systems of the Indo-Gangetic Plains: a synthesis of NARS-IRRI partnership research (No. 40). International Rice Research Institute.
- Lim SK, Son TW, Lee DW, Park BK and Cho KM (2001) Novel regenerated cellulose fibers from rice straw. Journal of Polymer Science 82(7): 1705-1708.
- Liu Z, Xu A and Long B (2011) Energy from combustion of rice straw: status and challenges to China. Energy and Power Engineering 3(03): 325.
- Lynd LR, Weimer PJ, Van Zyl, WH and Pretorius IS (2002) Microbial cellulose utilization: fundamentals and biotechnology. Microbiology Molecular Biology Review 66(3): 506-577.
- Matsumura Y and Yokoyama SY (2005) Current situation and prospect of biomass utilization in Japan. Biomass Bioenergy 29(5): 304-309.
- Mohammadi A, Cowie A, Mai TLA, de la Rosa RA, Brandão M, Kristiansen P and Joseph S (2016) Quantifying the greenhouse gas reduction benefits of utilizing straw biochar and enriched biochar. Energy Procedia 97: 254-261.
- Mondal SR, Rehana MJ, Noman MS and Adhikary SK (2010) Comparative study on growth and yield performance of oyster mushroom (*Pleurotus florida*) on different substrates. Journal of the Bangladesh Agricultural University 8(2):213-220.
- Murali S, Shrivastava R and Saxena M (2010) Greenhouse gas emissions from open field burning of agricultural residues in India. Journal Environment Science Engineering 52(4): 277-84.
- NAAS (2013) Climate Resilient Agriculture in India. Policy Paper No. 65, National Academy of Agricultural Sciences, New Delhi: p 20.
- Oanh NTK, Bich TL, Tipayarom D, Manadhar BR, Prapat P, Simpson CD, Liu LJS (2011) Characterization of particulate matter emission from open burning of rice straw. Atmospheric Environment 45(2): 493–502.
- Ookawa T, Inoue K, Matsuoka M, Ebitani T, Takarada T, Yamamoto T, Ueda T, Yokoyama T, Sugiyama C, Nakaba S and Funada R (2014) Increased lodging resistance in long-culm, low-lignin gh2 rice for improved feed and bioenergy production. Science Reporter 4: 6567.
- Saritha M, Arora A and Nain L (2012) Pretreatment of paddy straw with Trameteshirsuta for improved enzymatic saccharification. Bioresource Technology 104: 459-465.
- Sarkar N and Aikat K (2012) Kinetic study of acid hydrolysis of rice straw. ISRN Biotechnology Volume 2013, Article ID 170615.
- Sharma S, Kumar R, Gaur R, Agrawal R, Gupta RP, Tuli DK and Das B (2015) Pilot scale study on steam explosion and mass balance for higher sugar recovery from rice straw. Bioresource Technology 175: 350-357.



- Singh BP, Hatton BJ, Singh B, Cowie AL and Kathuria A (2010) Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. Journal Environment Quality 39(4): 1224-1235.
- Singh R, Srivastava M and Shukla A (2016) Environmental sustainability of bioethanol production from rice straw in India: a review. yJournal of Renewable Sustainable Energy 54: 202-216.
- Singh Y and Sidhu HS (2014) March. Management of cereal crop residues for sustainable ricewheat production system in the Indo-Gangetic Plains of India. In Proceeding of Indian National Science Academy 80(1): 95-114.
- Srinivasarao C, Gopinath KA, Venkatesh G, Dubey AK, Wakudkar H, Purakayastha TJ, Pathak H, Jha P, Lakaria BL, Rajkhowa DJ and Mandal S (2013) Use of biochar for soil health management and greenhouse gas mitigation in India: Potential and constraints. Central Research Institute for Dryland Agriculture, Hyderabad, Andhra Pradesh, p 51.
- Suramaythangkoor T and Gheewala SH (2010) Potential alternatives of heat and power technology application using rice straw in Thailand. Applied Energy 87(1): 128-133.
- Thiribhuvanamala G, Krishnamoorthy S, Manoranjitham VP and Krishnan S (2012) Improved techniques to enhance the yield of paddy straw mushroom (*Volvariella volvacea*) for commercial cultivation. African Journal Biotechnology 11(64): 12740-12748.
- TirolPadre A, Tsuchiya K, Inubushi K and Ladha JK (2005) Enhancing soil quality through residue management in a ricewheat system in Fukuoka. Japan Journal Soil Science and Plant Nutrition 51(6): 849-860.
- Tripathy A, Sahoo TK and Behera SR (2011) Yield evaluation of paddy straw mushroom (*Volvariellaspp*) on various lingo-cellulosic wastes. Building Research and Information 4(2):19-24.
- Utami CP and Susilawati PR (2017) Rice straw addition as sawdust substitute in Oyster mushroom (*Pleurotusostreatus*) planted media. AIP Conference Proceedings 1868, 090002. https:// doi.org/10.1063/1.4995194
- Wong ML, Webster EL, Spokes H, Phu P, Ehrhart-Bornstein M, Bornstein S, Park CS, Rice KC, Chrousos GP, Licinio J, Gold PW (1999) Chronic administration of the non-peptide CRH type 1 receptor antagonist antalarmin does not blunt hypothalamic-pituitary-adrenal axis responses to acute immobilization stress 165(4): 53-8.
- Yadvinder-Singh S, Humphreys E, Kukal SS, Brar NK (2005) Straw mulch, irrigation water and fertiliser N management effects on yield, water use and N use efficiency of wheat sown after rice. In ACIAR Proceeding 127: 171-181.
- Yang HS, Kim, DJ, Lee YK, Kim HJ, Jeon JY and Kang CW (2004) Possibility of using waste tire composites reinforced with rice straw as construction materials. Bioresource Technology 95(1): 61-65.
- Zhang Y, Ghaly AE and Li B (2012) Physical properties of rice residues as affected by variety and climatic and cultivation conditions in three Continents. American Journal Applied Science 9(11): 1759-1768.
- Zhu N (2007) Effect of low initial C/N ratio on aerobic composting of swine manure with rice straw. Bioresource Technology 31: 98(1): 9-13.\*



# Rice Mechanization in India: Key to Enhance Productivity and Profitability

## PK Guru, NT Borkar, M Debnath, D Chatterjee, Sivashankari M, S Saha and BB Panda

# SUMMARY

Rice mechanization contributes to sustainable increase in productivity and cropping intensity. Without a review of patterns and progress in farm power availability farmers would struggle to emerge from subsistence production. Present scenario of rice mechanization including availability of farm machinery/implements for different field operations starting from field preparation, sowing/transplanting, intercultural operations, harvesting, threshing and post harvesting operations is compiled. Future research areas are identified to enhance the mechanization level by performing field operations timely, precisely, and efficiently. Precise and need based application of inputs results in reducing cost of rice cultivation with optimal use of energy and drudgery reduction in farm operations.

# **1. INTRODUCTION**

Indian agriculture has made significant progress in the last five decades. However, past some years stagnating net sown area, reduction in per capita land availability, climate change and land degradation are posing serious challenges to it. India's population is increasing in a rapid rate which is likely to reach to 1.30 and 1.38 billion by 2020 and 2030, respectively (Goyal and Singh 2002). Rapid increase in population leading an immense pressure on Indian agriculture to produce more food in order to get food and nutritional security. On the contrary, the average land holding size is in a decreasing trend in India. The average estimated size of land holding in India would be mere 0.68 ha in 2020, and would be further reduced to a low of 0.32 ha in 2030. With all these challenges, India has the herculean task of ensuring food security for the most populous country by 2050. Hence, farming should effectively address local, national and international challenges of food, water and energy insecurity; issues related to climate change; and degradation of natural resources. Farm mechanization can play a key role in addressing these challenges for large as well as small-holder farmers.

Farm machinery and equipment provide a package of technology to (i) increase land productivity by improved timeliness of operations, reduced crop losses and improved quality of agro-produce; (ii) increase efficiency of inputs used through their efficient measurement and placement; (iii) increase labour productivity by using labour saving and drudgery reducing devices, and (iv) reduce cost of cultivation. Machinery is also important to harness available moisture at the time of tillage and sowing, hence dryland areas also experienced growth in farm machinery. Farm machines





like rotavator, ferti-seed-drill, raised bed planter and laser leveler boost water use efficiency of little water/moisture that is available; thereby enhancing productivity in dryland areas. There is a strong linear relationship between power available and agricultural productivity. Improved agricultural tools and equipment are estimated to contribute to the food and agricultural production in India by savings in seeds (15-20%), fertilizers (15-20%), time (20-30%), and labour (20-30%); and also by increase in cropping intensity (5-20%), and productivity (10-15%). International and national experiences have established the benefits of engineering inputs in terms of enhanced productivity by about 15% and reduction in cost of production by 20%, apart from increase in cropping intensity (20%), timeliness in farm operations and drudgery reduction.

Presently, India is the largest manufacturer of tractors in the world accounting for about one third of the global production. India also has a big network of agricultural machinery manufacturers. However, there is wide variation among the states at the level agricultural mechanization. The highest concentration of tractors is in northern India. After liberalization and with development of research prototypes of machines manufacturing got a big boost particularly in Haryana, Punjab, Rajasthan, Madhya Pradesh and Uttar Pradesh. Combine manufacturing is concentrated mainly in Punjab. About 700-800 combines are sold annually. Combine harvesting of wheat, paddy and soybean is well accepted by farmers in this area. The estimated levels of mechanization of various farm operations in India are: 40% for tillage, 30% for seeding/planting, 37% for irrigation and 48% for threshing of wheat, 5% for threshing of rest of the crops and 35% for plant protection (CIAE, Bhopal). There is close nexus between farm power availability and increased productivity. The productivity of rice in Punjab, Haryana and Western UP was more than other states namely Assam, Bihar, Jharkhand, eastern UP, Chhattisgarh, Odisha, and West Bengal as these states had farm power availability less than 1.50 kW per ha. The farm power availability in Indian agriculture and productivity increased from 0.25 to 1.84 kW ha<sup>-1</sup> and 0.52 to 1.92 t ha<sup>-1</sup>, respectively over the years from 1951 to 2012. The predicted values of farm power availability and productivity in India for the year 2020 is going to be increased to 2.2 kW ha<sup>-1</sup> and 2.3 t ha-1, respectively (Mehta et al. 2014). In farm mechanization level India is lacking behind other developing countries. Farm power availability in India is very low as compare to the level of mechanization of United State (95%), Western Europe (95%), Russia (80%), Brazil (75%) and China (57%) (Renpu 2014). One possible reason for India's low productivity in rice is the small size of individual farm holdings. The 2001 census found that 80% of farm holdings were less than 2 hectares in size, with 62% averaging less than half a hectare. Just 1% of the holdings was classified as large (over 10 hectares) and averaged 17.1 hectares. The overall average size of all holdings was only 1.33 hectares. A more recent government report noted that small farms have gotten even smaller, and that 85% of farmers lack access to farm inputs and credit. This is not surprising as the rural population has grown but the available farm acreage has not.

Farm mechanization is low in the rice-based farming systems in eastern India. However, it is picking up and many of the small and big farm-machineries are now a



common sight in eastern India. Even combine is also being used to harvest rice crop in some parts of eastern India. Over these years there was rapid shift in farm power uses from animal power to mechanical power. Mechanical power helps in timely farm operations with less labour and cost, but reduction in animal uses on farms increases the problem of crop biomass burning. In rice based cropping system managing rice straw is a big challenge. Complete machinery package is needed to be introduced to enhance the production and also it helps in minimize the input energy and cost involved in rice based cropping system. The use of machinery for field preparation operation for rice cultivation is high and most of the farmers of India are using tractor with matching implements for deep ploughing and puddling operation. But the further operation viz. sowing, transplanting, harvesting and threshing is done manually and having very low level of mechanization. In India, the availability of draught animals power has come down from 0.133 kW ha<sup>-1</sup> in 1971-72 to 0.094 kW ha<sup>-1</sup> in 2012-13, whereas, the share of tractors, power tillers, diesel engines and electric motors has increased from 0.020 to 0.844, 0.001 to 0.015, 0.053 to 0.300 and 0.041 to 0.494 kW ha<sup>-1</sup>, respectively during the same period. The rice transplanters market in India has grown from about 550 in 2008-09 to 1,500-1,600 units in 2013-14. The industry is expected to grow by more than 50 % in 2014-15 with Chhattisgarh, Odisha, Bihar and southern states showing positive sign of adoption of technology (Mehta et al. 2014). The objectives of the chapters are i) to study present status of farm machinery used in rice cultivation in India; and ii) to identify future research area to enhance rice mechanization in India.

# 2. PRESENT STATUS OF RICE MACHINERY IN INDIA

### 2.1. Tillage

On animal powered farms primary tillage is done by using Desi hal (Country plough), and MB plough. Animal drawn plough is still used for tillage in Himachal Pradesh, Assam, Bihar, UP, Odisha, West Bengal and Andhra Pradesh. Mostly these ploughs are manufactured by local craftsman and their design differs from place to place. Animal drawn disc harrow, spike harrow, spring type harrow, blade harrow, zigzag harrow, three and five tyne cultivators, clod crusher, chisel ploughs, sub-soilers, scraper, bund former and wooden leveler are commercially available. On tractor powered farms mould board plough (MB) and cultivators are two most commonly used implements. Mould board plough is used for primary tillage operation and cultivator

is used for primary as well as secondary tillage operations (Fig. 1). Tractor drawn disc harrows are popular for dry secondary tillage operation. Now rotavator is gaining popularity due to its capability for multiple



Fig. 1. Field preparation using tractor drawn MB plough (a) and cultivator (b).



operations in one time. Saving of 60-70 per cent in operational time and 55-65 per cent in fuel consumption with single rotavator compared to the conventional method of seed bed preparation with separate ploughing and harrowing operations have been observed, besides conservation of moisture due to destruction of capillaries.

### 2.2. Puddling

Puddling operation is performed to reduce deep percolation of water, to suppress weeds by decomposing them and to facilitate transplanting of paddy seedlings by making the soil softer (Fig. 2). In animal powered farms for puddling



Fig. 2. Puddling using tractor rotavator (a) and animal drawn disc harrow (b).

operation bullock drawn cono-puddlers, disc harrow-cum puddler, in power tiller or tractor powered farms power tiller mounted cono-puddler, power tiller rotavator and tractor drawn paddy disc harrow, cage wheel with cultivator and rotavator, are machinery used for puddling which are commercially available.

### 2.3. Land leveling

In India rice farmer's uses traditional land leveling and laser land leveling for final field preparation. Traditional land leveling includes animal drawn leveler or tractor or even bulldozers in the case of highly undulated land. The accuracy of these implements is low, which results in uneven distribution of irrigation water. Laser land leveling is an alternative to achieve higher level of accuracy





in land leveling. This gives uniform land for seed sowing or transplanting with uniform distribution of irrigation water (Fig. 3).

### 2.4. Seeding, planting and transplanting

Rice is grown either by direct seeding i.e. broadcasting, drilling in dry soil, sowing in wet soil or by transplanting. As per power availability (manual, animal, power tiller, and tractor) on farms there are plenty of sowing implements developed and most of them are for dry direct sowing of rice including one, two and three row manual seed drill, three row animal drawn seed drill, self propelled hill seeder, power tiller and tractor drawn seed drill (Fig. 4) for upland conditions for plain terrain whereas manual, bullock drawn and power tiller drawn seed drills are suitable for hilly terrain. For wet direct sowing of rice manual operated drum seeders are popular. The manual drum of 4-rows and 6-rows seeder being light in weight can be operated easily by female farm



Rice Research for Enhancing Productivity, Profitability and Climate Resilience

women in low land area. Manual drum seeders of 4, 6 and 8-rows are available commercially. For transplanting of rice manual operated transplanter and power operated transplanters are commercially available (Fig. 4).



Fig. 4. Tractor drawn seed cum fertilizer drill (a) and self propelled eight row transplanter (b) in operation.

### 2.5. Weeding

The weeds are more competitive with crops during the initial stages of their growth (2-6 weeks after planting). Controlling weeds during this time is very essential for realizing maximum crop yield. Manual uprooting of the weeds with hand in squatting

and bending postures is the common practice for wetland rice. For weeding in rice hand hoe, finger weeder, conoweeder animal drawn weeder, power tiller operated and self-propelled weeders are commercially available and used by the farmers (Fig. 5).

### 2.6. Fertilizer application

Mostly manual broadcasting of fertilizer is performed by farmers. However, for deep placement of fertilizers for higher efficiency of applied nutrient (mostly N), deep placement applicator are used (Fig. 6).



Fig. 5. Weeding in rice with two row wet land weeder (a) and single row dry land weeder (b).



Fig. 6. Deep placement of urea briquettes.

#### 2.7. Plant protection

Timely application of herbicides, pesticides and fungicides (collectively called Crop Protection Products) at peak periods play a vital role in ensuring better yields from a crop. The magnitude of this problem is further amplified due to shortage of labour during this time. Different types of duster and sprayers have been developed for operation by hand, a small engine, power tiller and also by using the tractor power source. For application of pesticides, the farmers most commonly use hand



compression sprayer, knapsack sprayers and power sprayers. Low volume and ultra low volume (ULV) sprayers, which require comparatively smaller quantity of water, are also in use.

### 2.8. Harvesting

For rice harvesting many technologies have been developed such as reaper, combine harvester etc. but in eastern India still manual harvesting using sickle is predominant method of paddy harvesting. It takes about 170-200 man hours to harvest one hectare of paddy. Improved sickles, walk behind self-propelled vertical conveyor reaper (Fig. 7), power tiller operated vertical conveyor windrower, animal drawn reaper, tractor rear mounted reaper windrower, tractor operated straw combine, reaper binder and combine harvester are available commercially. These harvesting equipments are being used by famers for harvesting of paddy for plain field on custom hiring basis.

### 2.9. Threshing

Threshing is one of the most mechanized operations in rice cultivation. On animal powered farms threshing by bullock treading is practiced on large scale in the country but it is also time consuming and involves drudgery. Pedal operated thresher is used for threshing of rice by the farmers of West Bengal, Odisha, Assam, Andaman, Bihar and Jharkhand states. The output capacity, threshing efficiency and labour requirement were 44 kg/h, 98.8% and 5 man-h/q, respectively. Power operated axial flow thresher (Fig. 7) works on axial flow principle. It consists of spike tooth cylinder, straw thrower, concave, sieve shaker and aspirator blowers. It is suitable for threshing

rice. It can be operated with power tiller, tractor, engine and electric motor. Axial flow thresher operated by single 1.5 kW motor/power tiller engine was developed for hilly region. The capacities of power tiller and tractor



Fig. 7. Threshing by power operated drummy thresher (a) and harvesting by reaper (b).

operated axial flow threshers are 3 to 5 q  $h^{-1}$  and 10 to 12 q  $h^{-1}$ , respectively. Pedal operated, animal drawn, power tiller operated, tractor drawn and electric motor operated threshers are commercially available and have become very popular for threshing operation in plain region.

### 2.10. Straw management machinery

Ministry of New and Renewable Energy (MNRE 2009), Govt. of India estimated that about 500 MT of crop residue is generated every year. The surplus residues i.e., the residues generated, in excess of the less amount of residues used for various



purposes, are typically burned in the field or used to meet household energy needs by farmers. Burning of crop residues leads to 1) release of soot particles and smoke causing human health problems; 2) emission of greenhouse gases (GHGs) such as carbon dioxide, methane and nitrous oxide causing global warming; 3) loss of plant nutrients such as N, P, K and S; 4) adverse impacts on soil properties and 5) wastage of valuable C and energy rich residues. There are several options which can be practiced such as composting, generation of energy, production of biofuel and recycling in soil to manage the residues in a productive manner. Generally, many farmers are keeping rice straw in form of heap for feeding to animals. In north-western (NW), India combine harvesting of rice and wheat is now a common practice, leaving large amount of crop residues in the fields. Rice straw incorporation is practiced by less than 1% farmers as it is energy and time-intensive. It is a common practice that crop residue is burnt directly in the field, causing environment pollution. It is an important challenge to manage the huge quantity of crop residue and its proper utilization. Conservation agriculture (CA) practices in North India offers a good promise in using these residues for improving soil health, increasing productivity, reducing pollution and enhancing sustainability and resilience of agriculture. The technologies (RCTs) involving no- or minimum-tillage, direct seeding, bed planting and crop diversification with innovations in residue management are possible alternatives to the conventional energy and input intensive agriculture. But most of the technologies are for rice- wheat cropping system. In eastern India in irrigated lands rice-rice cropping

system is dominant. Here farmers got paddy straw two times in a year and no energy efficient technology is used by the farmers. Paddy straw management machinery available in India is straw chopper cum spreader, happy seeder (Fig. 8), straw management system for combine harvester and baler.



Fig. 8. Happy seeder (a) and spatial till drill (b) in operation.

### 2.11. Water management in rice

To produce 1 kg of rice around 3000-5000 liters of water are often used which is more than any other crop like wheat and maize (Satyanarayana et al. 2007). Generally surface irrigation method, viz., check basin method is used for irrigating paddy crop. Unfortunately, the efficiency of surface irrigation in India varies from 30-40% where as in country like Australia the efficiency in surface irrigation ranges as high as between 60-85% (Burton 2016). Fresh water for irrigation is becoming increasingly scarce due to population growth, increasing urban and industrial development (Bouman 2007; Belder et al. 2005). Any measure contributing saving in irrigation water in rice will yield in large saving of water. Source of irrigation water may be either surface water or ground water. Over the past three decades ground water has become



the main source of growth in irrigated areas which at present accounts for over 60 per cent of the irrigated area in the country (Gandhi et al. 2009). Generally, internal combustion engine (IC) like diesel engines are used for pumping irrigation water in India. It is a costly affair also the NOX gases released during use of IC engine for irrigating crop has environmental pollution effect.

### 2.12. Post harvest management

Paddy after harvesting undergoes a series of processing operations to convert to an edible form. The edible portion of paddy is the rice. The various unit operations involved in the processing of paddy to rice include cleaning, drying, storage, parboiling (optional) milling and polishing. Care should be taken at each one of these unit operations to minimize the loss and maximize the head rice yield recovery. Use of modern rice mill with the improved unit operations and equipments gives higher return with improved quality. Spoilage in paddy occurs due to improper handling and storage practices. Moisture content of paddy plays a major role in maintaining the quality of the product. Recommended moisture content for harvesting is 20-25% and safe moisture content for storage of paddy for 2 to 3 weeks, 8 to 12 months and more than a year are 14-18%, 13% or less and 9% or less respectively. So, drying also plays a major role in controlling the moisture level, thereby in deciding the quality of the rice. Presently farmers are using open sun drying for drying of threshed paddy and after drying storage in soil bins or concrete silos.

Rice milling is the oldest and the largest agro processing industry of the country. Paddy grain is milled either in raw condition or after par-boiling, mostly by single hullers of which over 82,000 are registered in the country. Apart from it there are also a large number of unregistered single hulling units in the country. A good number (60%) of these are also linked with par-boiling units and sun-drying yards. Most of the tiny hullers of about 250-300 kg h<sup>-1</sup> capacities are employed for custom milling of paddy. Apart from it double hulling unit's number over 2,600 units, under run disc shellers cum cone polishers numbering 5,000 units and rubber roll shellers cum friction polishers numbering over 10,000 units are also present in the country. Further, over the years there has been a steady growth of improved rice mills in the country. Most of these have capacities ranging from 2 t h<sup>-1</sup> to 10 t h<sup>-1</sup>.

# 3. ICAR-NRRI DEVELOPED MACHINERY/IMPLEMENTS FOR RICE CULTIVATION

For rice cultivation improved farm implements/machines were developed by ICAR-NRRI Cuttack and popularized among farmers. These machines include bullock drawn implements, manual drawn implements, power tiller operated machines, self-propelled machines, weeding implements, transplanting implements, and post-harvest machines (Fig. 9 and 10). Most of the machines are low cost and suitable for marginal and small farmers. ICAR-NRRI developed farm implements/ machines for rice were commercialized through MOU with private manufacturers. Farm implements/machines were supplied to different states of the country. In last 10 years total 14 no. of farm implements/machines were commercialized and around 4000 number of units were sold.



Table 1. ICAR-NRRI developed machinery for rice mechanization.
--

Tillage implements	
Bullock Drawn two gang notch type disc harrow	Field Capacity- 0.35 ha h <sup>-1</sup> This implement can be used in light as well as heavy soils by increasing and decreasing the weight by filling sand inside the empty drum. Price- Rs. 20,000/-
Bullock drawn drum type disc harrow	Field Capacity- 0.4 ha h <sup>-1</sup> Used for light as well as heavy soils by increasing and decreasing the weight by filling sand inside the empty drum. Price- Rs. 20,000/-
Sowing implements	
One row manual seed drill	Field capacity- 0.01 ha h <sup>-1</sup> Saves 45 % time over manual seeding Gives uniform row to row spacing Price- Rs. 1500/-
Two row manual seed drill	Field capacity- 0.019 to 0.022 ha h <sup>-1</sup> Adjustable seed delivery rateuniform row to row space for weeding operation Price- Rs. 3000/-
Three row manual seed drill	Field capacity- 0.03-0.04 ha h <sup>-1</sup> Saving in seeds and labours in sowing of crops Easy weeding and inter-culture operation Price- Rs. 4000/-
Three row manual puddle seeder	Field Capacity- 0.15 ha h <sup>-1</sup> Adjustable seed rate, saving of seed and time of seeding Price- Rs. 3500/-
Four row manual drum seeder	Field capacity- 0.030-0.034 ha h <sup>-1</sup> Reduced seed rate by 60-65 % as compared to broadcast seeding. Price- Rs. 4500/-
Six row manual drum seeder	Field capacity- 0.04 ha h <sup>-1</sup> Reduced seed rate by 55-60 % as compared to broadcast seeding. Labour requirement in weeding in drum seeder plots was reduced by more than 70% due to use of mechanical weeders.
Eight row manual drum seeder (hyperboloid shape)	Price- Rs. 6500/- Field capacity- 0.093 to 0.097 ha h <sup>-1</sup> Sowing of this method reduced seed rate by 50-55 % as compared to broadcast seeding. Price- Rs. 8500/-
Power tiller operated Multicrop seed drill	Field Capacity- 0.15 ha h <sup>-1</sup> One hactare area can be easily covered in single day by an operator About 70-80 % labour saving over manual operated sowing implements Price- Rs. 20,000/-

Contd....



Power tiller operated seed drill for rice and groundnut	Field capacity- 0.15 ha h <sup>-1</sup> Different seed rates i.e. 30 to 100 kg/ha for rice and 40 to 135 kg/ha for groundnut could be achieved. Price- Rs. 22,000/-
Self-propelled eight row hill seeder	Field capacity- 0.25 ha h <sup>-1</sup> Reduced drudgery involved in sowing in puddled field condition Uniform seeds per hill (3-4)Labour saving by 60% over manual drum seeder application
	Price- Rs. 60,000/-
Transplanter	
Two row transplanter	Field capacity- 0.02 ha h <sup>-1</sup> Saving in labour by 40-45 % as compared to manual transplanting Gender friendly technology females can easily operate it
	Price- Rs. 6500/-
Four row transplanter	Field capacity- 0.03 ha h <sup>-1</sup> Saving in labour by 55-60 % as compared to manual transplanting Price- Rs. 8500/-
Deep placement urea briquet	
Two row urea briquette	Field capacity- 0.07 ha h <sup>-1</sup>
applicator	Labour requirement- 15 man-h/ha Price- Rs. 3500/-
Three row urea briquette applicator	Field capacity- 0.08 ha h <sup>-1</sup> Labour requirement- 12 man-h/ha Price- Rs. 4500/-
Injector type urea briquette applicator	Field capacity- 0.03 ha h <sup>-1</sup> Labour requirement- 40 man-h ha <sup>-1</sup> Price- Rs. 2000/-
Weeding implements	
Wheel finger weeder	Field capacity- 0.022 ha h <sup>-1</sup> Saving in labour by 40-50% as compare to manual weeding Price- Rs. 700/-
Finger weeder	Field capacity- 0.012 ha h <sup>-1</sup> Saving in labour by 27-30% as compare to manual weeding Price- Rs. 320/-
Star-Cono-Weeder	Field capacity- 0.013-0.017 ha h <sup>-1</sup> Saving in labour by 30-32% as compare to manual weeding Price- Rs. 1900/-
Single row power weeder	Field capacity- 0.025 ha h <sup>-1</sup> Saving in labour by 50-55% as compare to manual weeding Reduced drudgery during weeding operation Price- Rs. 22,000/-

Contd....



#### Post Harvest and Processing machinery

Power rice winnower cum	Capacity clean grain – 500 kg h <sup>-1</sup>
cleaner	Cleaning efficincy- 98 %
	Cost of operation Rs. 6 per quintal as compare to manual
	cleaning Rs. 16 per quintal
	Price- Rs. 20,000/-
Mini paddy parboiling unit	Capacity-75 kg batch <sup>-1</sup>
	Time required- 6 h per batch
	Cost of operation Rs. 30 per quintal as compare to manual
	Rs. 40 per quintal
	Price- Rs. 6500/-
Manual rice winnower	Capacity- 90 kg h <sup>-1</sup>
	Cost of operation- Rs. 10/quintal
	Price- Rs. 6000/-
Chaff and husk stove	It consumes 1.5-2.0 kg husk in one batch burns for 45 min <sup>-1</sup> hr
	Price- Rs. 800/-
Power operated paddy	Capacity- 3-4 q h <sup>-1</sup>
thresher	Threshing efficiency- 98.5%
	60% saving in labour requirement and 54% saving in cost of
	threshing as compared to those of pedal type paddy thresher



Fig. 9. NRRI developed three row seed drill (a), six row drum seeder (b) and power operated single row dry land weeder (c) in operation.



Fig. 10. Manual operated three row (a) and two row urea briquette applicators (b)



## 4. FUTURE THRUST AREA

#### 4.1. Mechanical rice transplanting

Transplanted paddy cultivation is considered to be better from crop management and productivity point of view. Farmers of India used traditional methods of rice transplanting involved more human work rather than use of advanced technologies. Mechanical transplanter is most prominent option to avoid drudgery and time involved during transplanting operation. For Indian conditions the present need is to mechanize the small holding transplanting operation by introduction of low cost mechanical transplanter. The transplanters are used for only limited period of 15-30 days in a year. Therefore, farmers do not want to invest large amount on costly machines. To reduce the cost and to overcome the problems associated with operation of manual transplanter there is need to develop a small self-propelled type transplanter. The transplanting mechanism and forward speed should be power driven and controlled by the operator, so operator only needs to guide the transplanter. The existing popular transplanters are needed to be modified for simultaneous application of urea in root zone of rice and to reduce the nitrogen losses from field. Development of mechanical transplanters for large mat type seedlings is needed so that it can be more popular on custom hiring basis and easily available for small and marginal farmers. Nursery seeder may be developed for sowing of paddy seeds in nursery trays. This gives the uniform seedling population in trays and also the soil media selected becomes easy for cutting by transplanter fingers and gives optimum seedlings per hill. Transplanter capable for working under adverse field conditions viz. standing water on fields, less prepared field, plant residue on surface, are needed to be developed. Root-wash type seedlings transplanters need to be developed, so that the need of mat type nursery can be eliminated. Precision transplanters can be developed for large farmers to save time and to reduce the input cost.

#### 4.2. Nitrogen management in rice cultivation

Urea, widely used nitrogenous fertilizer, is available in various forms like prilled urea, pelleted urea, briquetted urea etc. Among these various forms prilled urea are used in most of the cases. Owing to the problem of its larger surface area, only 30– 45% of the broadcasted prilled urea can be utilized by the plants and rest are lost in the various sinks into the environment (Dong et al. 2012). To avoid this loss, the urea is deep placed in reduced zone in rice field or slow release forms are used (e.g. supergranules, urea mudballs, briquettes, coated urea). Some advantages of deep placement using urea briquette applicator are: (a) higher yield and nitrogen use efficiency (b) reduces loss of nitrogen (c) reduce greenhouse gas emission (d) less labour cost (e) precise placement. Compared to the manual placement, use of machine for deep placement of urea briquette applicator can improve the placement and reduce manual errors in placement depth.

#### 4.3. Precision rice machinery

Developments in electronics, sensors, and information technology are now permitting considerable up gradation of farm machinery in terms of minimizing the wastage of inputs, reducing drudgery, improving the quality of farm produce and making agriculture more environment-friendly. The input applicators such as seeders/ planters, fertilizer applicators, agro-chemical applicators and irrigation systems need to take into account the spatial variability in the field while dispensing the precise quantity of inputs. The present technologies of sensors, remote sensing, electronics, and mathematical modelling permit their integration with machinery design to permit the precision input applicators' development. Development of pneumatic planter for precise seed rate, precision fertilizer applicator based on site specific requirement, precision mechanical transplanter, and variable rate chemical applicators need to be developed for rice crop.

#### 4.4. Paddy straw management machinery

To avoid burning of paddy residue, improved machinery need to be developed and commercialized. A series of implements were developed in North India but still these implements were not being used by the farmers. On-farm management of paddy straw machines can save time, maintain soil health and remove the need of multiple operations to be done to incorporate the paddy straw. Straw chopper, happy seeder, combine with straw management system, half feed combine etc. are technology available in the Indian market. These machines need to be tested and popularize in different areas of the country.

#### 4.5. Energy efficient machinery

Energy requirements in agriculture sector depend on the size of cultivated land, level of mechanization, cropping pattern, and climatic conditions. Climate change and environmental sustainability are the key issues, must be dealt with while producing more food grains under use of various energy resources. These issues can be checked through efficient utilization and conservation of energy at the most. Maximum benefits in agricultural production can be drawn through optimal and proper utilization of energy inputs involved in various farm operations available with farmers. As per the size of land holding and method of crop cultivation, selection of energy efficient technology is due important.

In present conditions, farm machinery available and used is mostly operated by non-renewable sources of energy (Fossil fuels). Limited fossil resources emphasizes the need for new sustainable energy supply options through use renewable energies. Solar farming is slowly getting popularity and stationary agricultural tools such as watering systems, dryers, green house etc. are available in market. There is a need to give more emphasize on standardization and popularization of these technologies. There is need to develop solar energy based farm machines/implements to replace the fossil fuel based engines. Use of solar energy and modern micro irrigation techniques in rice crop cultivation will help in increasing crop water productivity and yield besides reducing cost of cultivation.



In rice cultivation use of machinery for field preparation is high and most of the farmers of India are using tractor and power tiller with matching implements for deep ploughing and puddling operations. But, for other operations viz. sowing, transplanting, harvesting and threshing human labour or animals are used which leads to higher use of energy input in rice production. Optimal use of energy sources available in farm can reduce the input energy without affecting output. Based on the energy footprints of rice cultivation improved package of practices through inclusion of improved implements are recommended for cultivation of rice by different methods viz. DDSR, transplanting and WDSR (Table 2).

Farm type	Dry direct sowing	Transplanting	Wet direct sowing
Animal Farm/ small farm (less than 2 ha)	Bullock ploughing (MB Plough) x 1 + bullock disc harrow x 2; sowing by bullock drawn seed drill; weeding: chemical + mechanical + manual; FYM application by bullock cart; chemical spray by hand compression sprayer; harvesting by improved sickle; threshing by manual pedal thresher; transportation by bullock trolley	Bullock ploughing (MB Plough) x 1 + bullock disc puddler x 3; mat type nursery preparation; transplanting by manual transplanter; weeding: chemical + mechanical + manual; FYM application by bullock cart; chemical spray by hand compression sprayer; harvesting by improved sickle; threshing by manual pedal thresher; transportation by bullock trolley	Bullock ploughing (MB Plough) x 1 + bullock disc puddler x 3; sowing by Manual drawn six row cylindrical drum seeder; weeding chemical + mechanical + manual; FYM application by bullock cart; chemical spray by hand compression sprayer; harvesting by improved sickle; threshing by manual pedal thresher; transportation by bullock trolley
Mechanized farm (more than 2 ha)	Power tiller/ tractor - cultivator (2) + disc harrow (2); sowing with PT seed cum fertilizer drill/tractor drawn seed cum fertilizer drill; weeding with power weeder + manual weeding; manual fertilizer application; chemical spray by power sprayer; harvesting by reaper and threshing by power operated drummy thresher harvester; transportation by tractor trolley	Power tiller/tractor - cultivator (2) + Disc harrow (2); mat type nursery preparation; transplanting by power transplanter; weeding with power weeder + manual weeding; manual fertilizer application; chemical spray by power sprayer; harvesting by reaper and threshing by /power operated drummy thresher/ harvester; transportation by tractor trolley	Power tiller/ tractor- cultivator (2) + disc harrow (2); sowing with eight row cup type power seeder; weeding with power weeder + manual weeding; manual fertilizer application; chemical spray by power sprayer; harvesting by reaper and threshing by power operated drummy thresher/ harvesting by combine harvester; transportation by tractor trolley

## Table 2. Improved cultivation practices for direct sown rice cultivation for optimal energy use.



#### 4.6. Post harvest processing and value addition

Single pass rice mills with metal polisher need to be improved with rubber roll sheller for better performance. Rice parboiling and drying system need to be improved for better energy efficiency. Rice husk and straw can be properly utilized for energy generation. Community level improved methods for drying, cleaning, milling, and packaging can help in increase of farmer's income. Value added products of rice (Rice flour, puffed rice, rice flakes) have excellent commercial demands, to popularize these values added products low cost and user friendly technology needs to be developed.

## 5. WAY FORWARD

- Development of decision support system and expert system for precise input application and crop management for different agro-ecology regions of India
- Promoting establishment of more custom hiring centers with large machinery tractor, combines, thresher etc is essential to enhance the mechanization level for future of the industry.
- Testing facility of farm implements/machines need to be established in region specific to maintain the quality and ensure safety.
- Community level improved methods of drying, cleaning, milling, and storage of rice need to be promoted.

#### References

- Belder P, Spiertz JHJ, Bouman BAM, Lu G and Tuong T P (2005) Nitrogen economy and water productivity of lowland rice under water-saving irrigation. Field Crops Research, 93(2):169-185.
- Bouman BAM (2007) A conceptual framework for the improvement of crop water productivity at different spatial scales. Agricultural Systems 93(1):43-60.
- Burton M (2016) Water management issues at the farm level. Indian Irrigation Forum International Commission on Irrigation and Drainage (ICID) India Water Week.
- Dong NM, Brandt KK, Sørensen J, Hung NN, Van Hach C, Tan PS, and Dalsgaard T (2012) Effects of alternating wetting and drying versus continuous flooding on fertilizer nitrogen fate in rice fields in the Mekong Delta, Vietnam. Soil Biology and Biochemistry 47:166-174.
- Gandhi V P and Namboodiri NV (2009) Groundwater irrigation in India: Gains, costs, and risks. Indian Institute of Management Ahmedabad, Gujarat, India.
- Goyal SK and Singh JP (2002) Demand versus supply of foodgrains in India: Implications to food security. Paper presentation at the 13th International Farm Management Congress, Wageningen, The Netherlands, July 7-12, 2002, p. 20.
- Mehta CR, Chandel NS, and Senthilkumar T (2014) Status, Challenges and Strategies for Farm Mechanization in India. Agricultural Mechanization in Asia, Africa, and Latin America, 45(4):18-23.
- Renpu B (2014) Analysis of the Trends of Agricultural Mechanization Development in China (2000-2020). ESCAP/CSAM Policy Brief, Issue No.1, 9-11.
- Satyanarayana A, Thiyagarajan TM, and Uphoff N (2007) Opportunities for water saving with higher yield from the system of rice intensification. Irrigation Science, 25(2):99-115.\*





## Microbial Resources for Alleviating Abiotic and Biotic Stresses and Improving Soil Health in Rice Ecology

## U Kumar, P Panneerselvam, TK Dangar, A Kumar, D Chatterjee, C Parmeswaran, SD Mohapatra, G Prasanthi, K Chakraborty, P Swain and AK Nayak

## **SUMMARY**

Abiotic and biotic stresses decrease the rice productivity; hence, there is an urgent need to develop simple and low-cost microbial tools for management of these stresses. Beneficial microbes present in soil and plant have huge potentials to alleviate the stresses. We characterized few thermo/drought-tolerant microbes (Bacillus sp., Aspergillus oryzae etc.) to alleviate drought stress in rice and also developed biocontrol formulations of Bacillus thuringiensis, Beauveria bassiana, Metarhizium anisopliae and Skermanella sp. for managing rice leaf folder. We also explored the potential of Azolla as biofertilizer and livestock feed. Cylindrospermopsis sp. association in Azolla as abundant cyanobiont was first time documented which has enlightened to rethink about Azolla-based biofertilizer strategies. Further, nutrient profiling of different strains of Azolla suggested the prospects of Azolla microphylla as suitable livestock feed. The efficient straw decomposing microbes (*Bacillus*, Aspergillus, Trichoderma, and Streptomyces spp.) available with us, will be utilized to develop a consortium to decompose paddy straw within one month. To explore the beneficial role of Arbuscular mycorrhizal fungi and uncultivated microbial community and understanding their structural and functional variations in rice ecosystem through molecular approach are frontier research in future for sustainable rice production, particularly in Eastern India. Overall, the present chapter deals the prospects of beneficial microbes in alleviating abiotic and biotic stresses; improving nutrient use efficiency and managing rice residues.

## **1. INTRODUCTION**

Most of the cultivable lands around the world are severely affected by abiotic and biotic stresses which are evident with one report that for every 1°C rise in day/night temperature above 28/21°C declined the rice yield by 10%. Therefore, a wider range of adaptations and mitigation strategies would be required to meet the challenge of enhancing productivity of rice from affected lands. Beneficial microorganisms are one of the best options to alleviate these stresses in agricultural crops, therefore, we must explore the microbial potential particularly their unique properties of tolerance to extremities, ubiquity, genetic diversity, and their interaction with crop plants for sustainable rice production with higher yield stability (Grover et al. 2014; Kumar et al. 2017a).



Rhizosphere is the place where microbial communications interact with plant roots and effects higher numerical abundance and diversity of microbes compared to bulk soil (root-free soil). Several reports indicated that microbial population in the rhizosphere is highly influenced by differing physico-chemical and biological properties of soil (Kumar et al. 2016; Kumar et al. 2017b). Soil harbours vastly diverse of microbial communities, such as, bacteria, cyanobacteria (blue green algae), fungi and actinomycetes (Kumar et al. 2017c). Of these, bacteria are ubiquitous in soil, possibly because of having rapid growth rate and also have the ability to use a wider range of substrate as carbon and/or nitrogen sources.

Most of the soil bacteria are adhered to soil particles and heavily influenced by plant root exudates. Therefore, depending upon the nature of root exudates and soil condition, the population of bacteria fluctuate in the vicinity of plant root and their interactions are categorized into three types *viz.*, beneficial, harmful and neutral. Reports indicated that more than 80% bacteria are beneficial to the plants and these are of two types-1) symbiotic bacteria which have a mutual relationship with the plant, and 2) free-living bacteria in the soil, are often associated closely, on, or even within the roots of plants. Plant growth-promoting rhizobacteria (PGPR) is another term for beneficial free-living soil bacteria and sometimes referred as yield increasing bacteria (YIB). Three most intrinsic characteristic need to be possessed by PGPRs, namely, they must have ability i) to colonize the root, (ii) to survive, reproduce and expressing plant-growth functions in the microhabitats of root surface under influence of other microbiota, and (iii) finally, able to help in growth promotion of plant.

In general, application of PGPRs are commonly advocated in most of the crops for making efficient and sustainable nutrient availability in plants and these can be grouped in different categories based on their nature and function, such as, a) nitrogen-fixing microbes: *Azospirillum, Azotobacter, Rhizobium, Gluconacetobacter* spp., *Azolla*-cyanobiont (blue green algae) etc., b) phosphate solubilizing microbes; bacteria-*Pseudomonas striata, Bacillus megaterium, Bacillus subtilis* etc., Fungi-*Penicillium* sp., *Aspergillus awamori* etc., c) phosphorous mobilizing biofertilizers: Arbuscular mycorrhiza fungi-*Glomus, Gigaspora, Acaulospora, Scutellospora, Sclerocystis* spp. etc., d) silicate and zinc solubilizers: *Pseudomonas, Bacillus* spp. etc., e) potassium solubilizer: *Fraturia aurantia* etc.

Based on the above background, the present chapter emphasizes on: 1) microbemediated alleviation of drought and management of leaf folder under rice ecosystem, 2) status and research needs of *Azolla* as biofertilizer and livestock feed, 3) molecular understanding of *Azolla*-cyanobiont association, 4) role of Arbuscular mycorrhiza fungi (AMF) in rice and recent status of microbial consortia for paddy straw decomposition, and 5) abundance and diversity of different structural and functional genes related to biogeochemical cycling of major nutrients, such as, nitrogen (N), phosphorus (P) and potassium (K). The overall concept of whole chapter is represented in Fig. 1.



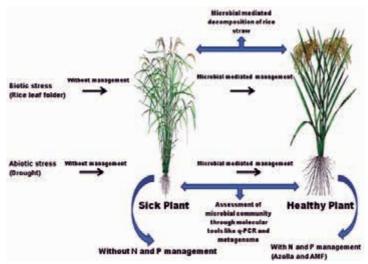


Fig. 1. Diagrammatic representation of overall viewpoints covered in this chapter a) microbe- mediated management of rice leaf folder, b) drought, c) paddy straw decomposition by microbes, d) role of AM fungi in rice, e) bioprospects of *Azolla* and its cyanobionts, and f) assessment of microbial community under rice ecosystem.

## 2. STATUS OF RESEARCH/KNOWLEDGE

#### 2.1. Microbe-mediated alleviation of drought/moisture stress in rice

Most of the agricultural and agronomic practices are designated to optimize crop growth by avoiding abiotic stresses (drought, saline, acidity, UV, nutrient etc.). Drought or moisture stress is one of the most important abiotic stresses which usually occurred due to the shortage of water. Plants are adversely affected by drought due to alteration of its physiological processes, leading to decline in crop yield. Drought tolerant plant-growth promoting microbes (PGPMs) is one of the alternatives to mitigate these problems. Researchers have demonstrated some of the mechanisms of PGPMs by which plants fight with drought or water stress. Table 1 represents few mechanisms of PGPMs which helps in alleviating drought and/or moisture stresses in agricultural crops including rice.

At ICAR-National Rice Research Institute (NRRI), Cuttack, we have attempted some of the activities related to alleviating drought stress by PGPMs in rice. In this connection, we screened a plant-growth promoting thermo-tolerant *Bacillus* sp. strain TBB1 from Tarabalo hot springs of Odisha and evaluated its efficacy in rice. The TBB1- treated plant showed better growth promotion in rice in terms of root length and dry weight at the higher temperature. Additionally, ten thermo-tolerant plant growth promoting fungi (PGPF) were also screened for PGP traits. Among them, the 6 potent thermo-tolerant PGPF were identified and submitted at National Center for Biotechnology Information (NCBI), New York, USA and National Fungal Culture Collection of India (NFCCI), Pune, India with accession numbers KJ652020-KJ652025 and NFCCI 3438 - NFCCI-3443, respectively.



Table 1. List of microbial genes responsible to alleviate drought or moisture stress in agricultural crops including rice (Adapted from Bal et al. 2012; Meena et al. 2017 and Shekhar et al. 2017).

Source	Host plant	Role of the gene
Bacteria	Rice, tomato, canola, tobacco etc.	Decreased level of ethylene under drought/moisture stress
Escherichia coli	Rice	Tolerant to drought
Paenibacillus polymyxa	Arabidopsis	Tolerant to drought
Rhizobium YAS34 strain	Sunflower	Tolerant to drought
Yeast	Tomato	Tolerant to drought
Bacillus subtilis	Tobacco	Tolerant to drought
Yeast	Melon	Tolerant to drought
Pleurotus sajor- caju	Tobacco	Tolerant to moisture stress
Escherichia coli	Peanut	Tolerant to drought
Arthrobacter globiformis	Tomato	Tolerant to drought
Escherichia coli	Cotton	Increased drought tolerance
Escherichia coli	Rice, arabidopsis and maize	Tolerant to water deficit
Bacillus subtilis	Rice, arabidopsis and maize	Tolerant to water deficit
	Bacteria Escherichia coli Paenibacillus polymyxa Rhizobium YAS34 strain Yeast Bacillus subtilis Yeast Pleurotus sajor- caju Escherichia coli Escherichia coli	BacteriaRice, tomato, canola, tobacco etc.Escherichia coliRicePaenibacillus polymyxaArabidopsisPaenibacillus polymyxaArabidopsisRhizobium YAS34SunflowerYeastTomatoBacillus subtilisTobaccoYeastMelonPleurotus sajor- cajuTobaccoPleurotus sajor- cajuTobaccoScherichia coliPeanutEscherichia coliCottonBacillus subtilisSine, arabidopsisBacillus subtilisRice, arabidopsis

### 2.2. Microbial formulations to manage rice leaf folder

The highest yield of any crop is based on the improved variety, appropriate pest, disease management, and recommended fertilization. Adequate pest management is essential for sustainable agricultural production. In the worldwide agriculture system, the commonly used pesticides come under synthetic origin, such as, carbamate, halogenated, organophosphorus etc. compounds. Excessive use of these synthetic compounds led to creation of new strains of resistant besides environmental pollution. Hence, biopesticides are considered as an alternative to synthetic pesticides that are highly effective, target specific and reduce environmental risks. Up to now, there are more than 3000 kinds of microbes are reported to cause diseases in insects. However, further research is required to find out the remaining undiscovered or unidentified



microorganisms that are used in insect pest management. So far, nearly one hundred bacteria identified as entomopathogens, among them *Bacillus thuringiensis* (Bt), *Metarrhizium* sp. and *Beauvaria* sp. have got the maximum importance as microbial control agents.

Rice is consumed by the one-third population of world, but the huge economic loss of this crop is due to attack by many insect pests. Annually, pest accounted the loss of more than 5% of the world total rice production, however, in India, the grain yield loss of rice is accounted in the range of 21–51% due to insect attack and the infestation varies depending on agro- climatic conditions. More than 100 species of insects attack on rice crop, among them, rice leaf folder (RLF), *Cnaphalocrocis medinalis* (Guenee) is considered as a serious insect pest. Earlier, the leaf folder was considered as a minor pest of rice, but recently its importance has been increasing in areas where modern high yielding varieties are grown. Managing leaf folder in rice is challenging task and among different strategies, biopesticides can play important role as a principal system to manage this pest.

Many microbial biocontrol agents have been documented, however, *B. thuringiensis* is considered as one of the desirable alternatives to chemical pesticides (Chatterjee et al. 2017). *B. thuringiensis* accounts for about 5-8% of total *Bacillus* sp. in the environment. Till date, more than 130 species of coleopteran, dipteran, and lepidopteran insects are found to be controlled by *B. thuringiensis*. So far, 71 serotypes (84 serovars) of *B. thuringiensis* having a wide array of host range have been commercially exploited directly as a native form or indirectly as transgenic microbes or plants. Bacteria, especially *B. thuringiensis* and *B. sphaericus* are the most potent and successful group of organisms for effective control of insect pests and vectors of diseases. Similarly, *Beauveria bassiana*, and *Metarhizium anisopliae* are reported to have strong biocontrol activity against rice leaf folder.

At NRRI, Cuttack, we are actively working since long to identify efficient entomopathogens to manage rice leaf folder and finally able to identify the following bacterial and fungal strains *viz., B. thuringiensis, B. bassiana* and *M. anisopliae*, and formulations of these strains were also filed for Indian patents. In addition, recently we have identified one efficient entomopathogenic bacterium (*Skermanella* sp.) against rice leaf folder and pink stem borer.

#### 2.3. Bio-prospects of Azolla for soil health improvement

*Azolla* is an aquatic fern and able to fix unlinked nitrogen ( $N_2$ ) directly from the atmosphere by endosymbiotic cyanobacteria and is thus a very promising supplier of nitrogen to aquatic ecosystems. It produces 10 to 20 t/ha/season of fresh biomass and supplies 20 to 40 kg N/ha and also has the ability to improve the low nitrogen use efficiency in rice (Yao et al. 2018). *Azolla* has several other important applications which include organic manure, bioremediation, livestock feed, human diets and most importantly in biofuel production. Therefore, to make *Azolla* technology very popular among farming community a unique approach (sporocarp-based formulation of *Azolla*) is required and further to resolve the controversial taxonomy of *Azolla*-cyanobiont association, molecular methods may be helpful to explore the potentialities of *Azolla*.



#### 2.3.1. Sporocarp-based formulation of Azolla

Azolla technology is widely accepted throughout the world as efficient nitrogen contributor in rice ecology through symbiotically associated cyanobacteria with them. Sporulation and sexual reproduction of *Azolla* are essential for the survival of the associations under adverse environmental conditions, such as tropical /sub-tropical summers and temperate zone winters. Field observations and laboratory studies indicate that sporulation in *Azolla* species is not simply a seasonal phenomenon linked to photoperiod, rather, sporulation appears to be induced by the interacting effects of environmental factors, including temperature, light intensity, nutrients, and plant density. Furthermore, the conditions leading to the induction of sporulation are not the same for all species (Brouwer et al. 2014).

As regards to the biomass production, and quantity of nitrogen fixation and nutrient recycling, *Azolla* is highly efficient, cost-effective and ecologically sound biofertilizer (Bhuvaneshwari et al. 2015). To produce *Azolla* inoculum in paddy fields, larger scale of its vegetative fronds are required but there are several physical constraints in *Azolla* production and utilization. The thick wall of megasporocarp can withstand high temperature, drought condition, and pest attack. That is why, many researchers were advocated to use sporocarp-based formulations of *Azolla* as biofertilizers, however, most of the researchers have documented the sporocarp production of *Azolla* only from a limited number of species, and therefore, it has to be studied thoroughly in species available at NRRI Cuttack germplasm collections.

Earlier researchers have documented the mass production of *Azolla* and their role in nutrient management in rice crop production at NRRI, Cuttack. They also have attempted on induction of sporocarp in different species of *Azolla* and found that some strains of *Azolla* are strongly sensitive to the environment and sporulate only during winter months (November to March).

#### 2.3.2. Bio-prospects of Azolla-cyanobiont

The available information on cyanobacteria association with *Azolla* is very poorly characterized; the degree of their interaction with the host, their mode of inheritance and patterns of co-evolution have not been fully investigated to date. Molecular tools are one of the best alternatives to characterize this phenomenon, but the whole genome sequence of *Azolla* is not yet available. Keeping the importance of *Azolla* in a huge applicability in agriculture and industry, there is an urgent need to generate *de novo* whole genome sequence data of *Azolla* to explore many possibilities. The genome size of *Azolla* is tiny (750 Mb) as compared to other pteridophytes genomes (>10 Gb) (Li and Pryer 2014), making it the perfect first genome to be sequenced in pteridophytes. *Azolla* is a very good source of protein and hence, it may consider as one of the important sources for cattle feed. Further, very limited reports are available on value-added products and renewable energy from *Azolla*.

Most of the reports available on *Azolla*, pertaining to its importance as biofertilizer and livestock feed. Nutrient profiling of *A. microphylla* was studied and its digestibility as feed supplement in livestock was also evaluated using *in vitro* technique. Studies



also showed the abiotic stress tolerance response of *Azolla* against salinity and ultraviolet radiations (UVB). Reports also indicated that *A. caroliniana* might be used as a sorbent for removing toxic substances from paper mill effluent (Shivkumar et al. 2015).

At NRRI, Cuttack, we are maintaining one hundred and two strains of *Azolla* germplasms, we did nutrient profiling of six species of *Azolla (A. pinnata; A. filiculoides; A. rubra; A. microphylla; A. mexicana* and *A. caroliniana*) and found that they all have higher percentage of nutrient value (protein, antioxidants, iron, calcium etc.) and least concentration of antifeedant value (acid and neutral detergent fibres) which indicated that *Azolla* can be used as potential livestock feed (Kumar et al. 2015). Furthermore, an Illumina-Miseq based *Azolla*-cyanobiont association in six species (name mentioned elsewhere) of *Azolla*, was also characterized which revealed for the first time that *Cylindrospermopsis* sp. was the most abundant *Azolla*-cyanobiont and their diversity was dependent upon presence of *Azolla* fibre content. Besides this, biomass production and mass multiplication of *A. microphylla* along with biochemical constituents under various conditions were also analyzed.

#### 2.4. Microbe-mediated paddy straw decomposition

In India, we are generating nearly 158 million tonnes of paddy straw every year and recycling of these wastes properly, retrieve the considerable amount of nutrients to the soil in addition to improving soil health and reducing greenhouse gas emission to the environment. It has been frequently reported that the application of rice straw to paddy fields increases methane emissions. Therefore, promotion of the oxidative decomposition of rice straw in and out of the field is important for not only reducing methane emissions but also enhancing the carbon stock in the soil.

Many research findings documented that the rice straw can be decomposed by using the combination of microbes like a bacterial and fungal consortium. In rice field, it was observed that application of decomposed rice straw decreases methane emission (Takakai et al. 2017). In composting, bacteria play a major role as they make up 80-90% of microorganisms found per gram of the compost. They can easily grow on soluble substrates and have the capacity to attack more complex materials by releasing extracellular enzymes. They contribute to a major proportion (80% of the total microbial count) in compost and are responsible for degradation of a variety of organic materials by releasing a wide range of enzymes. They are also responsible for the initial decomposition.

Sannathimmappa et al. (2015) reported that treatment of rice straw with a combination of cow dung slurry (5%), *Trichoderma harizianum* (5 kg/ha) and *Pleurotus sajor-caju* (5 kg/ha) showed significant degradation of rice straw. Researchers also reported that nutrient enriched compost can be prepared by using the consortium of fungal (*Aspergillus nidulans*, *A. awamori*, *T. viride* and *Phanerochaete chrysosporium*) inoculants within 70-90 days by pit and windrow methods. In general, organic wastes are rich in lignin content which makes the composting process a little challenging to the microbes for the degradation because



of its complexity to degrade as well as it reduces the bioavailability of other cell wall constituents. Some researchers have reported that fungi belonging to basidiomycetes group are good at degrading lignin. Similarly, actinomycetes are found to grow abundantly during the later stages of composting. Most frequently occurring actinomycetes during the later stages of composting include *Micromonospora*, *Streptomyces*, *Nocardia*, and *Thermoactinomyces*. The development of microbial consortium based on bacteria, fungi and actinobacteria would be more effective for decomposition of paddy straw than individual genus.

We have isolated nine strains from paddy straw and composting pit and evaluated for their ability for decomposition of rice straw under microcosm experiment. The results indicated that actinobacteria isolates (DA10, DA13, and DA9) degraded the cellulose 72.7 to 81.6% higher than uninoculated control after one month of inoculation, whereas, fungal (DF15, DF7 and DF19) and bacterial isolates (DB12, DB20 and DB23) showed the cellulose degradation efficacy by 66.6 and 79.0% higher, respectively compared to control. The bacteria, fungi and actinobacteria were identified as *Bacillus*, *Trichoderma*, and *Streptomyces* spp., respectively.

### 2.5. Role of Arbuscular mycorrhizal fungi (AMF) in rice

Arbuscular mycorrhizal fungi (AMF) are key components of soil microbiota and it comes under phylum Glomeromycota which represents the most common and widespread in terrestrial plant symbiotic association. The AMF is one of the most beneficial microbes for agricultural crops because of its contribution in soil structure, plant nutrition, disease resistance, drought and salinity tolerance (Auge et al. 2015). Rice crop planted under upland area willingly associated with mycorrhizal colonization but under lowland area or flooded conditions, colonization is infrequent due to the anoxic environment. However, recent studies conducted at NRRI, Cuttack showed that some of the species of AMF could colonize well under wetland condition of rice (Sahoo et al. 2017).

AMF colonization in rice plant has been documented by Sahoo et al. (2017) and this fungal association in rice found to enhancing P acquisition. The above findings clearly indicated that the AMF association in rice plant is essential for plant growth improvement. Plants acquire Pi by two mechanisms; i) directly via root epidermal cells and root hair ii) through the extra-radical hyphae of AMF which delivers Pi directly to colonized cells in the root cortex. It is well known that Pi is transported into plant cells through membrane- associated Pi transporter proteins belonging to Pht family. In rice, sequencing of rice genome has revealed 13 such phosphate transporter genes. In AMF colonized rice roots, Pht 11 and Pht 13 exhibited altered expression. Pht 11 is activated through symbiosis between AMF and rice, whereas, Pht 13 is independent which is expressed both in AMF colonized roots and uncolonized roots. So, Pht transporter protein Pht 11 is crucial for rice-AMF colonization. The phosphate transporter sociation (Glassop et al. 2007).

At NRRI, Cuttack, AMF association was studied in 72 different rice cultivars including two low P tolerant checks *viz.*, Kasalath and Dular, which were raised in P



deficient soil (< 6.0 - 8.0 ppm). The AMF root colonization was recorded in the range of 20-90%, whereas, it was 80-90% in Kasalath and Dular cultivars. These two varieties have the dominant unique type of vesicle-forming AMF colonization, which was not observed in many low P tolerant varieties. This observation clearly indicates that some genera of AMF may prefer the specific rice genotype of rice. It is well-known fact that Kasalath and Dular possess the protein kinase gene *pstol* for phosphorus-deficiency tolerance, thus these varieties having a unique kind of AMF root colonization in P deficient soil, needs further in-depth investigation.

## 2.6. Abundance and diversity of structural and functional genes related to biogeochemical cycling of nitrogen, phosphorus and sulphur under rice ecosystem

Among all nutrient cycles in the earth, nitrogen (N), phosphorous (P) and sulphur (S) are found to be dominant and soil microbes are playing a vital role to complete their cycles. Assessment of these microbes are tedious because only small percentage are cultivable and majority of them are undetectable, therefore, molecular techniques are to be used to monitor microbial populations in a variety of environmental samples at concentrations previously considered undetectable because this technique bypass cultivation dependent approach. Quantitative PCR (q-PCR) is one of the most important molecular tools widely used in microbial ecology nowadays to assess quantification of structural and functional genes within a complex community (Church et al. 2005) or to monitor their gene expression (Zehr et al. 2007). In addition to q-PCR, a metagenomic approach is also considered as a powerful molecular technique to assess culture independent profiling of microbial communities in complex ecosystem including rice.

At NRRI, Cuttack, culture based soil and plant microbial communities in rice have been studied under influence of different management practices such as influence of nitrogen fertilizers (Kumar et al. 2017a), non-target effect of chlorpyrifos (Kumar et al. 2017b) and even frequency of diazotrophs in aromatic rice cultivars (Kumar et al. 2017c), however, very limited study has been conducted to observe shift of microbial community in NPS biogeochemical cycling at genetic level.

## 3. KNOWLEDGE GAPS

Based on the above information, we observed following problems which need to be addressed in upcoming days.

- Water shortage is one of the major problems for rice cultivation and to mitigate this problem, a plenty of information has been generated under *in vitro* but adequate understanding and behavior of drought-tolerant microbial formulations are lacking under *in vivo* condition.
- Though, we have many microbe-based biocontrol agents, however no effective and consistent biocontrol agents are available till date for management of rice leaf folder.

- At present, the sporocarp-based primary inocula in place of vegetative propagules is essentially required for *Azolla* large-scale mass production, but this technology is not successful among farming community due to various reasons like time bound sporulation frequency, extreme temperature, variable photoperiod, low light intensity, soil pH, EC etc. These problems have to be resolved for making successful sporocarp-based *Azolla* inoculum.
- In addition, the method of storage, self-life, mode of delivery of sporocarp-based *Azolla* inocula are to be standardized.
- To date, whole genome sequence of *Azolla* is not available, hence it is difficult to understand cyanobacteria-based nitrogen fixation process in *Azolla* and biomass production under rice-based cropping system.
- Limited superior strains of *Azolla* are available for huge biomass & bioenergy productions, as well as, suitable feed for livestock.
- No report are available about the exact biochemical pathway of *Azolla* cyanobacterial-interactions and also systematic molecular approach to identify different metabolite production in *Azolla*.
- Many *ex situ* composting technologies are available and it requires 75-90 days for complete decomposition of paddy straw, but we do not have any viable *in situ* rice straw decomposing methods within short period of time (45-60 days).
- Though, the AMF associations have been reported in many crops, the information on rice is inadequate. Furthermore, it was reported that the plant growth performance and nutrient uptake will vary from species to species, hence, there is a need for selection of efficient AMF for better plant growth and development. Some reports also indicated that AMF has host, as well as, ecological preference, but this information in rice is not clear so far.
- In rice ecosystem, adequate information on abundance of soil microbial structural (*16S rRNA, ITS, AML*) and nutrient mobilizing functional genes (*nifH, amoA, nirK, nosZ, phoD, soxB* etc.) are not yet to be exactly quantified.

## 4. RESEARCH AND DEVELOPMENT NEEDS

Thermo-and drought-tolerant exopolysaccharide (EPS) producing plant growth promoting bacteria and fungi are available at NRRI Microbiology laboratory and these strains must be thoroughly characterized for different functional genes (acdS, ostA/B, sacB, hal1, tp, tps1, mtdl, codA/coxA,cspA/B, betB etc.) related to drought stress and efficient one must be formulated and evaluated under *in vitro* and *in vivo* conditions in rice ecosystem to make a suitable microbial product which can alleviate drought stress in rice.





Six entomo pathogens strains (four *B. thuringiensis* and one each of *B. bassiana* and *M. anisopliae*) were evaluated under laboratory and glass house conditions for management of leaf folder and filed patent also. But these strains should be validated for their efficacy and mode of delivery for managing rice leaf folder at farmers' field. In addition, bio-safety data should also be generated before commercialization. If we develop an efficient bio-formulation for management of leaf folder, it will be one of the potential components in organic cultivation of rice and also considerably reduce insecticides usage in rice cultivation.

Though, the technology of *Azolla* as biofertizer is available for the farmers, but not gaining much popularity in rice farming community due to huge inoculums requirement (2-5 t/ha) in the form of vegetative inocula. Hence, the sporocarp-based formulations will considerably reduce the initial inocula load to few liters per hectare, if we successfully exploit the sexually propagated inocula of *Azolla*. Sporocarp-based formulations will also ascertain the purity of strain which is not in case of earlier methods of application. Sexually propagated inocula (megasporocarp) can withstand adverse environmental conditions like high temperature, drought and pest attack.

Quantification of *nif* gene is one of the best alternatives to assess nitrogen fixing ability of *Azolla* under various environmental conditions. Metagenome and transcriptome sequencing of *Azolla* are other solutions to understand the co-evolution pattern of *Azolla*-cyanobiont. Identification of cost-effective protein rich *Azolla* stains for livestock feed is certainly needed to safeguard livestock health.

Straw burning problem has given a clue to develop the compatible lignocellulolytic microbial consortium for rapid decomposition of straw residues. The development of viable *in situ* partial or complete microbial decomposition of rice straw at filed levels will retrieve the considerable amount of nutrients to soil, improves soil qualities, decrease the greenhouse gas emission, suppress the population of stem borer and soil borne pathogens in rice cultivation. When the farmer realizes the benefit of *in situ* decomposition of rice straw in short periods (45-60 days), they will not burn the rice straw.

To mitigate P deficiency, AMF colonization with rice roots can be treated as a positive strategy for sustainable rice production. To unlock the potential of AMF for sustainable rice farming, identification of key molecular players involved in the colonization will open new vistas to design rice-AMF combinations with enhanced AMF performance.

For better understanding and generating knowledge towards microbial role in rice ecosystem, standardization of q-PCR protocol must be required to quantity different structural (*16S rRNA, ITS, AML* etc.) and functional (*nifH, amoA, nirK, nosZ, phoD, soxB* etc.) genes related to biological functions in paddy soil (Table 2). Finally, structural and functional variations of microbial community under influence of rice varieties, fertilizers and pesticides etc. are also essentially required for in-depth understanding of microbial role under rice system.



		Microbial group/		
Microbial genes	Primers	enzymes	Cycle	Reference
16S rRNA	F968/R1378	Total bacteria	-	Heuer et al. 2008
ITS	FF390/FR1	Fungi	-	Vainio et al. 2010
act	Act-878-a-a-19/ Act-235-a-S-20	Actinomycetes	-	Bredholt et al. 2008
ps	Ps-F/Ps-R	Pseudomonas	-	Anuj et al. 2009
nif H	NifH-F/NifH-R	Nitrogenase reductase	Ν	Rosche et al. 2002
amoA (Bacteria) amoA (Archaea)	AmoA-F/ AmoA-R	Ammonia monooxygenase	Ν	Hayden et al. 2010
nirK	NirK-F/NirK-R	Nitrite reductase	Ν	Yoshida et al. 2009
nosZ	NosZ-F/NosZ-R	Nitrous oxide reductase	Ν	Chon et al. 2011
phoD	PhoD-F733/ PhoD-R1083	Alkaline phosphatase	Р	Ragot et al. 2015
soxB	SoxB 710-F/ SoxB 1184-R	Sulphur oxidation	S	Tourna et al. 2014

 Table 2. Genes used for structural microbial community assessment and biological functions of agricultural soil.

## 5. CONCLUSION AND WAY FORWARD

Overall, the present chapter describes the different scenario of harnessing microbial resources for soil, pest and residue management. The following microbe-mediated works are essentially needed in future for sustainable development of rice crop particularly in Eastern India.

- Microbial formulation and methodology are to be developed to alleviate drought stress under rice cultivation system.
- The microbial consortium package should be developed exclusively for rice crop residues and pest management.
- Sporocarp-based formulations are to be developed to reduce the initial inocula load of *Azolla* in paddy field.
- Molecular markers are to be identified through meta-and transcriptome sequence of *Azolla* for better understanding of *Azolla*-cyanobiont interactions.
- Latest molecular tools must be explored to understand the soil biological nutrient cycling in paddy soil.

In conclusion, we urgently need a proper scientific research with technological developments to meet the target of food and feed security for the increasing world population. To achieve this goal an interdisciplinary approach is needed among scientists in different fields. As the microbes are ubiquitous in nature so that harnessing their diversified and pivotal potential provides an economical alternative for the development of climate-resilient crops. To address the above-said task a global



approach is a prerequisite to understand all the distinctive properties of microbes and it can be harnessed to fulfill the elevated demand for food, feed, and shelter. In the present era of scientific advancement, it is very feasible to integrate different advanced techniques in genomics and omics (transcriptomics, proteomics, metagenomics, metabolomics etc.) for development of viable technology. Most importantly, the longterm efficacy of stress tolerance and impacts associated with the use of microbes in genetic engineering of plants need to be assessed thoroughly. Extension of recombinant plant and microbial protocols would facilitate the validation process and the development of stress-tolerant crops. The long-term and in-depth studies on plant-microbe interactions are still needed to mitigate the elevated food and feeder demand in the era of global climate change.

#### References

- Anuj SN, Whiley DM, Kidd TJ, Bell SC, Wainwright CE, Nissen MD and Sloots TP (2009) Identification of *Pseudomonas aeruginosa* by a duplex real-time polymerase chain reaction assay targeting the *ecfX* and the *gyrB* genes. Diagnostic Microbiology and Infectious Disease 63:127-131.
- Auge RM, Toler HD and Saxton AM (2015) Arbuscular mycorrhizal symbiosis alters stomata conductance of host plants more under drought than under amply watered conditions: a meta-analysis. Mycorrhiza 25: 13-24.
- Bal HB, Das S, Dangar TK and Adhya TK (2012) ACC deaminase and IAA producing growth promoting bacteria from the rhizosphere soil of tropical rice plants. Journal of Basic Microbiology 53: 972–984.
- Bhuvaneshwari K and Singh PK (2015) Response of nitrogen-fixing water fern *Azolla* biofertilization to rice crop. 3 Biotech 5: 523-529.
- Bredholt H, Fjærvik E, Johnsen G and Zotchev SB (2008) Actinomycetes from sediments in the Trondheim fjord, Norway: diversity and biological activity. Marine Drugs 6: 12-24.
- Brouwer P, Bräutigam A, Külahoglu C, Tazelaar AO, Kurz S, Nierop KG, Werf A, Weber AP and Schluepmann H (2014) *Azolla* domestication towards a biobased economy? New Phytologists 202: 1069-1082.
- Chatterjee S, Azmi SA, Ghosh TS and Dangar TK (2017) Characterization of the *Bacillus thuringiensis* isolates virulent against rice leaf folder, *Cnaphalocrocis medinalis* (Guenee). In Proceedings of Zoological Society, Springer India 70 (1): 74-80.
- Chon K, Chang JS, Lee E, Lee J, Ryu J and Cho J (2011) Abundance of denitrifying genes coding for nitrate (*narG*), nitrite (*nirS*), and nitrous oxide (*nosZ*) reductases in estuarine versus waste water effluent-fed constructed wetlands. Ecological Engineering 37: 64-69.
- Glassop D, Godwin RM, Smith SE and Smith FW (2007) Rice phosphate transporters associated with phosphate uptake in rice roots colonized with Arbuscular mycorrhizal fungi. Botany 85: 644-651.
- Grover M, Madhubala R, Ali SZ, Yadav SK and Venkateswarlu B (2014) Influence of *Bacillus* spp. strains on seedling growth and physiological parameters of sorghum under moisture stress conditions. Journal of Basic Microbiology 54: 951-961.
- Hayden HL, Drake J, Imhof M, Oxley AP, Norng S and Mele PM (2010) The abundance of nitrogen cycle genes *amoA* and *nifH* depends on land-uses and soil types in South-Eastern Australia. Soil Biology and Biochemistry 42: 1774-1783.

- Heuer H, Focks A, Lamshöft M, Smalla K, Matthies M and Spiteller M (2008) Fate of sulfadiazine administered to pigs and its quantitative effect on the dynamics of bacterial resistance genes in manure and manured soil. Soil Biology and Biochemistry 40: 1892-1900.
- Kumar U, Kumar A, Umakanta N, Bagchi TB, Mohanty S, Kumar A, Shahid M and Nayak AK (2015) Nutrient profiling and identification of genetic marker for *Azolla* sp. In XXIII International Grassland Congress-IGC 2015 held on 20-24 November 2015 at Delhi NCR, India. http://www.internationalgrasslands.org/files/igc/publications/2015/1076.pdf.
- Kumar U, Banik A, Panneerselvam P and Annapurna K (2016) Lower frequency and diversity of antibiotic-producing fluorescent pseudomonads in rhizosphere of Indian rapeseed-mustard (*Brassica juncea* L. Czern.). Proceedings of National Academy of Science of India, Section B: Biological Science. https://doi.org/10.1007/s40011-016-0792-1.
- Kumar U, Shahid M, Tripathi R, Mohanty S, Kumar A, Bhattacharyya P, Lal B, Gautam P, Raja R, Panda BB, Jambhulakar NN, Shukla AK and Nayak AK (2017a) Variation of functional diversity of soil microbial community in sub-humid tropical rice-rice cropping system under long-term organic and inorganic fertilization. Ecological Indicators 73: 536-543.
- Kumar U, Berliner J, Adak T, Rath PC, Dey A, Pokhare SS, Jambhulkar NN, Panneerselvam P, Kumar A and Mohapatra SD (2017b) Non-target effect of continuous application of chlorpyrifos on soil microbes, nematodes and its persistence under sub-humid tropical rice-rice cropping system. Ecotoxicology and Environmental Safety 135: 225-235.
- Kumar U, Panneerselvam P, Govindasamy V, Vithalkumar L, Senthilkumar M, Banik A and Annapurna K (2017c) Long-term aromatic rice cultivation effect on frequency and diversity of diazotrophs in its rhizosphere. Ecological Engineering 101: 227-236.
- Li FW and Pryer KM (2014) Crowdfunding the *Azolla* fern genome project: a grassroots approach. Giga Science 3 (16):1-4.
- Meena KK, Sorty AM, Bitla UM, Choudhary K, Gupta P, Pareek A, Singh DP, Prabha R, Sahu PK, Gupta VK and Singh HB (2017) Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. Frontiers in Plant Science 8: 1-25.
- Ragot SA, Kertesz MA and Bünemann EK (2015) *phoD* alkaline phosphatase gene diversity in soil. Applied and Environmental Microbiology 81: 7281-7289.
- Rosch C, Mergel A and Bothe H (2002) Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. Applied and Environmental Microbiology 68: 3818-3829.
- Sahoo S, Panneerselvam P, Chowdhury T, Kumar U, Afrin J, Ansuman S and Anandan A (2017) Understanding the AM fungal association in flooded rice under elevated CO<sub>2</sub> condition Oryza 54: 290-297.
- Sannathimmappa HG, Gurumurthy BR, Jayadeva HM, Rajanna D and Shivanna MB (2015) Effect of paddy straw based integrated nutrient management practices for sustainable production of rice. IOSR Journal of Agriculture and Veterinary Science 8: 74-77.
- Shekhar S, Gambhir G and Padaria JC (2017) Bioprospecting of genes from microbes for stress management in agricultural crops. In agro-environmental sustainability, Springer International Publishing pp: 127-147.
- Sivakumar D and Nouri J (2015) Removal of contaminants in a paper mill effluent by *Azolla* caroliniana. Global Journal of Environmental Science and Management 1: 297-304.
- Takakai F, Ichikawa J, Ogawa M, Ogaya S, Yasuda K, Kobayashi Y, Sato T, Kaneta Y and Nagahama KI (2017) Suppression of CH<sub>4</sub> emission by rice straw removal and application of bio-ethanol production residue in a paddy field in Akita, Japan. Agriculture 7: 21.



- Tourna M, Maclean P, Condron L, O'callaghan M and Wakelin SA (2014) Links between sulphur oxidation and sulphur-oxidising bacteria abundance and diversity in soil microcosms based on *soxB* functional gene analysis. FEMS Microbiology Ecology 88: 538-549.
- Vainio EJ, Korhonen K, Tuomivirta TT and Hantula J (2010) A novel putative partitivirus of the saprotrophic fungus *Heterobasidion ecrustosum* infects pathogenic species of the *Heterobasidion annosum* complex. Fungal Biology 114: 955-965.
- Yao Y, Zhang M, Tian Y, Zhao M, Zeng K, Zhang B, Zhao M and Yin B (2018) Azolla biofertilizer for improving low nitrogen use efficiency in an intensive rice cropping system. Field Crops Research 216: 158-164.
- Yoshida M, Ishii S, Otsuka S and Senoo K (2009) Temporal shifts in diversity and quantity of *nirS* and *nirK* in a rice paddy field soil. Soil Biology and Biochemistry 41: 2044-2051.\*



## Exploring New Sources of Resistance for Insect Pest and Diseases of Rice

## M Jena, PC Rath, AK Mukherjee, Raghu S, GP Pandi G, Basana Gowda G, Prasanthi G, MK Yadav, MS Baite, Prabhukarthikeyan SR, MK Bag, S Lenka, Aravindan S, NB Patil, SD Mohapatra, Annamalai M and T Adak

## **SUMMARY**

In the present scenario of rice pest management, host plant resistance is playing a pivotal role. It is the easiest way to reduce pesticide load in rice ecosystems as well as to mitigate pest problem under adverse environmental conditions. With the development of nitrogen responsive high yielding rice varieties and hybrids, several insect pests and diseases assumed major status. Significant effort has been made in the past to develop resistant varieties against different pests, particularly, brown plant hopper, gall midge, blast and bacterial blight with identified resistant genes. But gradually, most of the developed varieties have lost their resistance resulting in more frequent and severe pest outbreaks. Therefore, again the search has began for new resistant donors of different pests from the existing vast germplasm collection of the country including those identified in the past. The mechanism of resistance in identified genotypes has to be studied by assessing its effect on pest biology and behavior or disease infection pattern. Molecular marker analysis of resistant genotypes (donors) will give an indication of the presence of a known or new gene effective against the particular pest. A set of donors should always be in place to be utilized in resistance breeding programme for the development of pest resistant varieties, which will benefit rice farmers of the country.

#### 1. Introduction

Productivity of rice is facing severe threat from biotic stresses, particularly insect pests and diseases, causing huge yield loss. Among the biotic stresses, insect pests like yellow stem borer (YSB), gall midge, brown plant hopper (BPH), white backed plant hopper (WBPH), and leaf folder are the major problems whereas among the diseases, blast, brown spot, bacterial blight (BB) and sheath blight (ShB) are most destructive to the crop. Recently, insects and diseases like case worm, swarming caterpillar, mealybug, gundhi bug, false smut, bakanae and sheath rot have emerged as the major problem in many rice growing areas of the country. Stored grain pests also are gaining significant importance in causing post harvest losses among which the rice weevil and angoumois grain moth are considered to be the most common pests. Moreover, rapid change in the virulence pattern of plant pathogen/insect populations pose continuous threat to existing popular rice varieties as well as for development of a virulent pathotype or biotype. Pesticide application has been followed by farmers as the major option for pest management which in turn, is creating



environmental pollution as well as health hazards by contaminating the food chain. Host plant resistance has the potential to be an alternative for effective, economic and ecofriendly means of pest management in rice independent of pesticide use. The resistance of rice plant to biotic stresses may be an inherited one (Resistant through donor) or it may be induced by application of an elicitor, activating the defense mechanism of the plant.

Resistant sources/donors are most important as they are the key to success of resistance breeding for developing pest resistant varieties. Keeping in view of the emerging pest problems in rice and the role of resistant donors in controlling these pests, the main objective should be to identify resistant donors against insect pests and diseases from the vast gene pool of the country. This can be achieved by evaluating large number of genotypes through standard screening techniques, supported by study on their mechanism of resistance. Mechanism of plant resistance against insects can be assured by biochemical analysis of the plant for its resistance-imparting contents such as sugar, silica, phenol *etc.* as well as antixenosis, antibiosis and tolerance studies. The resistance mechanism against diseases is to be ascertained through study of virulence patterns. Molecular characterization of identified resistant donors is necessary to look for the presence of resistant genes using reported markers or identification of new genes, if any. In the case of induced resistance, identification of effective elicitor/s is the prime need for inducing resistance in a popular but susceptible rice variety.

#### 2. Host plant resistance

Plant resistance is an inherited characteristic of a host that lessens the attack of a pest, may it be the insect, disease, nematode or other organisms. As per the recent pest management strategy through IPM, the use of resistant varieties should be the major or syncing with other control measures. Host plant resistance provides an efficient, economical, ecologically acceptable and safe means of crop protection. Resistant cultivars are the most durable, economical and practical means of tackling pest problems, being compatible with all other components of IPM. Besides constitutive resistance, the plant resistance to pests can also be induced either by endogenous or exogenous signaling molecules. Silicon, chitosan, plant growth promoting rhyzobacteria (PGPRs), jasmonic acid (JA), jasmonoyl-isoleucine (JA-Ile), salicylic acid (SA) and ethylene (ET) are some of the well known elicitors inducing plant to put forth its resistance. These exogenous materials or inducers are not directly toxic or inhibitory to pests but enable the plant to increase its level of resistance. Though several donors have been identified in the past against several insects and diseases and many varieties were developed by utilizing them, most of these donors as well as varieties have lost their resistance in the present day scenario either due to continuous exposure to the pest or due to development of more virulent population. Screening data of released resistant varieties at National Rice Research Institute (NRRI) gave an indication of gradual breaking down of their resistance.

The genotypes, still retaining the resistance are not popular among farmers due to low yield or cooking quality or taste or are not utilized to develop new varieties due to



their poor combining ability. Hence, identification of donors resistant to different rice pests, should be a continuous process to keep enough of such genotypes in store so that the development of pest resistant varieties will be sufficient and continuous to combat the pest situation in rice successfully. Further, the future challenge is to exploit the elicitors of induced defense in rice for pest management especially for borer pests. There is also a need to test the effect of foliar application of silicon on natural enemy foraging and impact. The research should focus on studying the integration of cultivar resistance and cultural controls, especially soil silicon amendment, against the entire pest complex in rice.

## 3. STATUS OF RESEARCH

## 3.1. Host plant resistance against insect pests

**3.1.1. Brown plant hopper:** The International Rice Research Institute, Philippines initiated studies on varietal resistance to BPH in 1966 with efficient mass- screening techniques. The basic technique has been introduced in Japan, Korea, Taiwan, India, Thailand, Sri Lanka, Indonesia and Solomon Island. The BPH resistant selections, IR 26 and IR 1561-228-3, were grown widely in the Philippines during 1974 and 1975. Both cultivars have Bph1 gene for resistance. IR36 and IR38 which have the bph2 gene for resistance to BPH were released by the Philippine Government during 1976. Varieties ASD 7 and Mudgo were resistant in the Philippines, Japan, Korea, Taiwan, Thailand and Indonesia, but susceptible in India. The different reaction is due to biotype formation. Biotype 1 in Southeast Asia (Philippines, China, Japan, Korea, Malaysia, Taiwan, Thailand); Biotype 2 in Philippines, Solomon Island, Vietnam; Biotype 3 in Southeast Asian countries (Philippines, Taiwan); Biotype 4 in South Asian countries of India, Bangladesh and Sri Lanka are widely distributed. Afterwards, though many resistant gene/QTLs were identified in different genotypes (Fujita et al. 2013), most of them have already lost their resistance against BPH population of India.

Following extensive damage by BPH during mid 70s, breeding for resistance was intensified in India which culminated in release of varieties like Jyothi in Kerala, Sonasali, Vajram, Chaitanya in AP, Neela and Udaya in Orissa and Manasarovar across the country. Some common donors were Ptb 33, Manoharsali, Rasi, Ptb 10, Ptb 20 etc. Afterwards, many BPH resistant varieties were released for different ecologies all over India and were released in different states (Krishnamurthy et al. 1995). By 2006, more than 65 varieties were released with resistance/tolerance to the insect. But, most of these varieties were developed keeping higher yield as the primary criteria and were later found to be resistant/tolerant to BPH. Therefore, in most cases after few years, the resistance breaks down and leading to the BPH menace. Several highly resistant donors have been identified at NRRI, Cuttack through green house screening according to the Standard Evaluation System during 2000-2016.

At NRRI, three thousand rice genotypes from NRRI Genetic resources were screened for their resistant reaction to BPH under greenhouse condition during the





years 2001 - 2016. More than 80 genotypes were found highly resistant with score 1. Some of them are – CRRI accession numbers 35677, 35703, 34997, 35003, 35927, 35070, 35155, 35183, 35184, 35228, 35181, 34969, 34993, 34997, 35014, 38448, 38448, 38449, 38450, 38452, 38459, 38469, 38500, 38530, 38552 and 38552C. One hundred lines, evolved from resistance breeding for BPH in the background of resistance from Salkathi (CR.Ac.35181) and Dhobanumberi (CR.Ac.35184), 10 lines were highly resistant with score '1' whereas 36 lines showed resistance of score '3'. The lines are : CR 2711-76, CR CR2712-22711-114, CR 2711-139, CR 2711-149, CR2712-2, CR 2712-11-1, CR 2712-11-13, CR 2712-229, CR 2713-8 and CR 2714-2, out of which CR2711-114, CR 2711-76, 2711-139, CR 2711-149 and CR2712-2 were found highly resistant (Jena and Sahu, 2013). Out of seventy entries from IRRI screened at NRRI, IR69726-29-1-2-2-2, IR70454-144-1-1-3-2 and IR72894-35-2-2-2 were found resistant. Out of 220 released varieties, IR-64 MAS and Hazaridhan scored '1' whereas CR-1980-1, Lalat MAS and Satyakrishna showed resistance of score '3'. Six hundred farmers' varieties of Odisha were evaluated and 21 were highly resistant (Jena et al. 2006; 2015).

Molecular approach for BPH resistance was initiated at NRRI with a DBT sponsored network project on "Identification and Functional Analysis of Brown Planthopper Resistance Genes in Rice". Under the programme, mapping population was developed from the cross TN1/Salkathi. Phenotyping was made of 300 RIL populations under artificial infestation conditions and the putative QTLs associated with the resistance to BPH were identified (Mohanty et al. 2017).

The line CR2711-76 is also found to be resistant to multiple pests of rice (AICRIP Report 2012-13) and having the resistant gene Bph 31 (Prahalada et al, 2017). Three accessions of IRRI Philippines, IR 73382-80-9-3-13-2-2-1-3-B (IR 64 x *O. rufipogon*), IR 75870-8-1-2-B-6-1-1-B (IR64 x *O. glaberrima*) and IR77390-6-2-18-2-B (IR69502-6-SRN-3-UBN-1-B x *O. glaberrima*) were found highly resistant against NRRI population of BPH . After being used in popular varieties like MTU 1010 and Swarna, three lines each from F3 lines of MTU1010/Swarna x IR 75870-8-1-2-B-6-1-1-B were highly resistant against BPH. Four genotypes from Assam Rice Collection (ARC), ARC - 333, 356, 11324 and 11309 were also found highly resistant. Two accessions of doubled haploid lines were highly resistant.

**3.1.2. White backed planthopper:** In general white backed planthopper (WBPH) has not received as much research attention as BPH. However, 14 loci have been identified for WBPH resistance. Since, it mainly occurs as a mixed population with BPH, identification of single resistant donor is as important as identification of common resistant donors for both. At IRRI, two resistant genes, *Wbph7 and Wbph8* has been identified from *O. officinalis.* Four varieties, IR48, IR52, IR60 and IR62 were reported as moderately resistant. More than 300 cultivars resistant to WBPH have been identified and 80 of them have been analyzed geneticlly (Brar and Khush, 2009). Four donors (N22, ARC 10239, ADR 52 and Podiwi A8) have been identified at IRRI and used to develop hopper resistant varieties. Several genes/QTLs have been identified for resistance to the pest (Fujita et al. 2013).



In India, *O. officinalis, O. punctata,* and *O. latifolia* showed high levels of resistance to the pest. IR 2035-117-3 has been used in breeding programmes for *S. furcifera* resistance (Padmavathi et al. 2007). Antibiotic Mechanisms of resistance to *S. furcifera* for reduction in feeding on the resistant cultivar Rathu Heenati could be attributed to the presence of certain water-soluble inhibitors in the plant. Low chlorophyll, low sugar, low amino acid and high phenol content in the plant had contributed for the resistance.

At NRRI, 167 rice genotypes from NRRI rice genetic resources and Punjab Agricultural University (PAU) were screened under net house condition. The genotypes IR 64 and TN1 were used as resistant and susceptible checks, respectively. The entries IC568061, AC 111, AC 1066, AC 1073, AC 124, AC 1418 were found highly resistant with score '1' whereas 1552(2) and 1552(8) from PAU, IC567998, IC568060, IC568065 and IC568082 were of score '3'. From 65 accessions reported resistant earlier against WBPH at NRRI, only four accessions, i.e., AC 34222, AC 34264, AC 38468 and AC 42425 were highly resistant at present against WBPH.

**3.1.3. Yellow stem borer:** Out of 17000 accessions screened against YSB at IRRI upto 1987, only about 40 *O. sativa* accessions and 80 wild rice accessions were found to have resistance against yellow stem borer. From India, CO 7, CO 15, CO 21, Ratna, TKM 6 and WC 1263 were identified as donors. Efforts were made to develop resistant/ tolerant varieties by using donors such as TKM6, CB 1, CB2 but none were good combiners to produce desired level of resistance. Still, some accessions showed moderate level of resistance which needs further evaluation. TKM 6 was found tolerant against striped stem borer, *Chilo suppresalis* and was utilized in breeding programme. The cultivars IR 36 developed at IRRI and Ratna developed at NRRI were having highest level of resistance.

Thousands of entries have been screened at NRRI against yellow stem borer (YSB). But most of them succumbed to the pest after continuous exposure for 2-3 years. One tropical japonica line WC-152 and a doubled haploid line SS-5 showed zero SES score against YSB in consecutive two years of screening against the susceptible check TN1 with damage score of 5. The inherent capacity of the pest to adopt a variety after 2-3 years exposure as evident from the screening data of NRRI for the past several years has not left avenue till now for choosing a highly resistant donor. Seven hundred and ten rice cultivars from NRRI that were evaluated against YSB in 2007, resistant cultivars with score 1 (below 5% white ear head) were NDR 402, CR 580-5, LPR 256, LPR 85, LPR 14, LPR 96-10, LPR 56-49, LPR 50, Kariawa 4, TCA 12, Bazail 65, Nali Hazara, Janaki, OR 1358-RGA-4, OR 1529-28-2, TKM 6, ARC 10660, Litipiti, Daonara, Chadhei Nakhi, Dahijhil, Brahmanbojni, Mahalakshmi, Jogen, Punshi, Triveni and Saket-4 as against Jaya the susceptible check with score 9 (28.1% WEH). Those are to be screened again for confirmation of resistance.

**3.1.4. Gall midge:** Wide spread cultivation of some of the resistant varieties carrying a single resistance gene has led to evolution of virulent populations, known to as biotypes, that are capable of overcoming the resistance. Existence and emergence of





new and virulent biotypes of the rice gall midge resulted in the breakdown of resistance in many of the popular gall midge resistant varieties. So far, 11 resistance (R) genes (designated Gm1 to Gm11) have been identified from different rice varieties (Himabindu et al. 2010). Seven distinct gall midge biotypes, differing in their virulence against these R genes have been reported (Vijaya Lakshmi et al. 2006). Using three or four of these sources of resistance, more than 60 gall midge-resistant rice varieties have been developed and released for commercial cultivation since 1975 (Bentur et al. 2003). Improved rice varieties carrying Gm1 or Gm2, however, have lost their resistance against gall midge in most of the rice growing areas. Exceptionally, varieties deriving resistance from Ptb21 have displayed resistance against five of the seven biotypes as per AICRIP report, 2006. Keeping this in view, thorough screening of various available rice germplasm is necessary in order to get new source of resistance.

Systematic evaluation of germplasm by NRRI for gall midge resistance during the 1950s - 1970s at hot spots such as Cuttack and Sambalpur in Odisha and at Warangal in Andhra Pradesh under field conditions resulted identification of sources such as Eswarakora, Ptb 18, Ptb 21, Siam 29, and Leuang 152. Some accessions of wild species of Oryza such as O. brachyantha, O. coarctata (now Porteresia coarctata), O. eichingeri, O. granulata, and O. ridlevi were reported to be gall midge resistant. None of the donors displayed resistance against all the six biotypes. Only Orumundakan was resistant against 5 biotypes. Ptb 27, Dhanala 27, Ptb 18, Ptb 21, ARC 5959 and 22 other accessions were reported resistant at NRRI during 1964, 1965 and 1974. Also studies prior to 1975 suggested the prevalence of biotype 1 at NRRI as differentials W1263 (with the Gml gene) and Leuang152 (Gm2) were resistant. Subsequently, this population evolved into biotype 2 by acquiring virulence against resistance conferred by the Gm1 gene. The present study shows resistance of W1263 whereas phalguna and ARC 5984 were susceptible. In recent years, all the known gene differentials of gall midge having resistant genes 1, 2, 3, 4, 5, 6, 7, 9, 10 and 11 showed susceptible reaction to NRRI gall midge population (Biotype 2), either showing breakdown of resistance or indicating a population change which is a researchable issue.

**3.1.5. Leaf folder:** The rice leaf folder has recently emerged as an important insect pest of rice in many Asian countries under changed climatic scenario. Management of the pest with insecticides is becoming costly and there are reports of resurgence. So, there is a need for the development of resistant cultivars to minimize the yield losses. In India, the varieties like GEB 24 (a mutant of Konamani) and TKM6, Ptb 33 were reported as resistant to leaf folder (Punithavalli et al. 2013). Similarly, TKM6, GEB24, CO7, PTB33, ARC10982, Shete, Bir-Me-Fen, Kaohsiung Sen Yu 169, *O. rufipogon* and *O. brachyantha* were reported as resistant genotypes. However, Ishaq A. (2014) observed that Kaohsiung Sen Yu 169 was susceptible, TKM6, Ptb 33 and GEB24 were moderately susceptible and only ARC10982 was moderately resistant against leaf folder. Likewise, Genotypes like CR 56-17 and its donor parents GEB-24, CR 190-103 and 294-548,Bundei, Harisankar, Sunakathi, Surjana, Juli and Sana chinamala and Chandanpedi were identified as resistant varieties at NRRI during 1985 to 1998. Genotypes such as RP 1746-1770-209; RP 2542-179-298; ARC 11281; RP 2543-136-



277; RP 2572-3-340; RP 2572-5-342; RP 2572-24-7 were relatively less susceptible while IR 8, IR 5 and TN 1 were highly susceptible. Three hundred sixty entries screened for resistance against leaf folder under field condition showed AC 42738, IC 569085, IC 569017 with less than 1% leaf damage and Konark, Rudra and Mahalakshmi had less than 5% damage against 12.5% leaf damage in susceptible check TN1.Using the biotechnological tools, two transgenic rice lines, expressing Cry1Ab, CpTI and Cry1Ac showed significantly lower damage at different developmental stages. Further, studies on mechanism of resistance need to be conducted to confirm either a tested rice variety/line is resistant or susceptible to the pest.

**3.1.6. Stored grain pests:** There is common belief that the aromatic fine rice varieties are more susceptible to insect attack. Khan and Halder (2012) reported Kalijira, an aromatic fine rice variety (locally known as polao rice), to be less infested by the rice weevil. The degree of susceptibility of the rice varieties from the highest to lowest in terms of number of adult insects was Lata (755) > Nazersail (695) > Minicate (654) > Pariza (482) > Kataribhog (456) > Kalijira (402). Parboiled rice varieties were reported to be more susceptible to stored pest infestation (Islam 2007).

At ICAR-NRRI, out of 20 rice varieties screened against *S. cerealella* multiplication, only Annada showed tolerant reaction upto 90 days. Other varieties like Heera, Kalinga III, Vandana, Sattari, Sneha, Dhaula, Naveen, Jaya, Indira, Saket-4, Tara, Kalinga III, Pooja, Panidhan, Pusa Basmati-1, Basmati 370, Durga and Ratna were susceptible and Tapaswini was highly susceptible to storage insects. So far many scientists in different countries have sorted out countless varieties of cereals resistant against *S.cerealella* to incorporate useful information in breeding programme.

**3.1.7. Induced resistance:** Silicon amendment conferred resistance to the rice leaf folder (Han et al. 2015), by increased leaf abrasiveness against *Spodoptera exempta* and *Schistocerca gregaria*. Nanosilica and Jasmonic acid were found to be effective in controlling the pests like army worm (Stout et al. 2009). Soil incorporation of Si and K present in fly ash mitigated the incidence of the rice stem borer, soil application of Si reduced the percentage of white head caused by *Chilo partellus* in Parto cultivar (Hosseini et al. 2012) and enhanced plant resistance to BPH (Yang et al. 2017). The induced resistance by JA did not produce any phytotoxicity (Senthil-Nathan et al. 2009). Methyl Salicylate (MeSA) at 100 mg L<sup>-1</sup> exhibited greater mortality against rice leaf folder (Kalaivani et al. 2017).

## 3.2. Host Plant Resistance against Diseases

**3.2.1. Blast:** Yang et al. (2017) screened 358 rice varieties for the presence of 13 major blast resistance (R) genes against *M. oryzae* using functional markers out of which 259 varieties were having one to seven R genes. Twenty-six SSR markers associated with blast resistance in a set of 276 indica landraces from China and few from different parts of the world were reported. Genome wide association mapping (GWAS) identified 16 LAFBR and 20 resistant cultivars with seventy-four candidate genes, which encode receptor-like protein kinases, transcription factors, and other defense-related proteins (Zhu et al. 2016). The identified markers associated with blast resistance can be





validated for their effectiveness in a variety of genetic backgrounds and can be helpful in the pyramiding of QTLs from different sources through marker-assisted selection.

The genetic diversity for eight resistant genes against rice blast, was assessed in landrace collections of Manipur, India and the presence of six to seven genes in rice accessions from the North Eastern state of Manipur was related to high level of resistance (Mahender et al, 2012). The rice blast resistant *Pi9* gene was analyzed in 47 rice germplasm using the dominant STS marker 195R-1/195F-1 derived from the Nbs2-Pi9 candidate gene and only six were positive for the Pi9 gene (Imam et al. 2013). Molecular screening of *Pi2* gene was carried out on 61 landraces of rice using gene based marker NBS2P3 and NBS2R derived from Nbs4-Pi2 candidate gene that generates a monomorphic band of 1.8 kb. Restriction digestion of PCR product with *EcoR1* enzyme, however, revealed polymorphism between susceptible and resistant lines. Out of 61 landraces, only five landraces had *Pi2* gene type banding pattern. The five landraces positive for *Pi2* gene are from Sikkim and Jharkhand (Alam et al. 2015).

Phenotyping followed by genetic diversity study at NRRI, of eighty rice varieties released by the institute, has been completed using molecular markers linked to twelve major blast resistance (R) genes *viz*. Pib, Piz, Piz-t, Pik, Pik-p, Pikm Pik-h, Pita/Pita-2, Pi2, Pi9, Pi1 and Pi5. Out of which, nineteen varieties (23.75%) showed resistance and twenty one were moderately resistant (Yadav et al. 2017). Among the 1314 germplasm accessions (ICAR-IIRR, NBPGR) evaluated for leaf blast resistance at Hazaribagh, 19 accessions (IC no. 245865, 246277, 246403, 246274, 454167, 121865, 199562, 218270, 245927, 246012, 246228, 246273 and 246659) were highly resistant (SES scores 0, 1, 2).

**3.2.2. Bacterial blight:** The most effective resistance gene Xa 21 was reported from wild rice by Ikeda et al. (1990), which was effective against all the races of Xoo in India. Long-term cultivation of rice varieties carrying single resistance gene has resulted in a significant alter in pathogen-race frequency and consequential breakdown of resistance. An example of this is failure of Xa4 which was integrated widely in many high yielding varieties of rice via conventional breeding. Extensive cultivation of varieties carrying *Xa4* has lead to the predominance of *Xoo* races that can overcome resistance conferred by this gene. One concrete solution to resistance breakdown is pyramiding of multiple resistance genes in the background of modern high yielding varieties. More than 36 resistance genes have been identified and designated in a series from *Xa1* to *Xa41* till now. The effectiveness of *R* genes varies over locations due to geographical structuring of the pathogen.

*Oryza barthii* found to have resistance against most of the races of Xoo in India especially in the Eastern India. Works at NRRI, reported BR-4-39-51-2, BR-51-49-6, IR3796-14-2, ARC-5925 & ARC 5943 as highly resistant and another 50 lines as resistant to BLB kresek phase. A total of 5000 lines were screened for bacterial blight resistance and 50 were resistant. Some of them are AC 36797, 35799, 36370, 36362, 35720, 36357, 36253, 35734, 36369, 35719, 35740, 36283, 35714 and 36294.

**3.2.3. Brown spot:** Three QTLs, qBS2, qBS9 and qBS 11 had been identified against the disease in cultivar Tadukan with latest qBS 11 having major effect (Sato et al.



2008). Satija et al. (2005) identified 15 *Oryza sativa* entries out of 124 classified as resistant (less than 5% severity). Conversely, Hossain et al. (2004) identified one resistant line out of 29 entries. Goel and Bala (2006) evaluated 219 wild rice accessions with diverse origin belonging to 15 *Oryza* species encompassing all the six genomes for their resistance to brown leaf spot and found that 15 lines were resistant while 78 lines were moderately resistant.

Synthesis of polyphenols and their oxidation products were observed to be associated with the phenomenon of resistance. A high correlation has been existing between the levels of phenols, lignin, flavonoids, silicon and oxidative enzymes in rice leaf tissue and the susceptibility of rice lines Giza 180 and Arabi to brown spot. However, Katara et al. (2010) identified 10 QTLs, some of which may be common to those of Sato et al.

Work at NRRI revealed that, Out of 573 Assam rice collections (ARC) screened against brown spot disease of rice, only 22 accessions were found moderately resistant. Those are ARC – 5846,5918, 5956, 5550, 6017, 6058, 6101, 6110, 6170, 6622, 7080,7335, 10618, 10670, 10922, 10934, 11206, 11434, 11566, 11641, 11679 and 12006.

**3.2.4. Sheath blight:** Many workers employed various methods for testing varietal resistance against this disease including (i) field tests using artificial inoculation (ii) seedling test (iii) inoculation at different stages of plant growth (iv) tests in pots and (v) sheath inoculation test. The results showed that, tall varieties with a few tillers were more resistant than short varieties with many tillers and resistant genes were located in tall varieties. In comparison with field ShB evaluation, the controlled chamber or mist-chamber assays were simple, precise and more reliable methods in tagging sheath blight resistance. Two wild rice species viz. *Oryza australiensis* and *O. nivara* were resistant against *R. solani*. Among different red (*Oryza sativa*) and wild rice (*O. alta, O. lativa, O. grandiglumis* and *O. glubepatula*) populations, none were found resistant (Santos et al. 2002).

Work at NRRI revealed that, A tolerant donor CR 1014 has been identified for disease, which has been utilized for developing mapping populations and transferring the tolerance gene to mega variety 'Swarna'. So far, out of 604 farmer's varieties from different parts of Odisha were screened by found that 31 varieties were found moderately resistant, 56 varieties showed tolerant reaction. A total of 90 NRRI released varieties were screened and 14 were moderately resistant and 16 were tolerant. Farmers varieties such as Biradia Bankoi, Dhusara, Ganjamgedi, Kalaketiki, Panikoili, Rajamani-K, Latamahu, Kendrapara-Kalama, Pasakathi, Tulasimali, Gangabhalu, Kandhamal-Jhalaka, KanakChampa, K-Balisara-LaktiMarchi, LaxmiVilash, Magra-P, Bolangir-Baidipali-Mahipal found moderately resistant/tolerant and NRRI varieties CR-1014, IR 64 MAS, CR Dhan-306, CR Dhan-601, CR Dhan-701, Kalinga-III, Satyakrishna, Gayatri, Reeta, Utkalprava, Geetanjali, Hanseswari, Binadhan 8, Varsadhan, Wita 9 found moderately resistant/tolerant. During 2012-13, 17 genotypes namely, Tapaswini, Annapurna, Swarna, Swarna sub 1, Tetep, CR 1014, ADT 39, Manasarovar, IET 19346, IET 20443, IET 19790, IET 20252, IET 17885, IET 17886, IET 19140, IET 20755 and IET 20216 were screened against the disease and results showed one genotype, CR 1014



as moderately resistant, four genotypes Tetep, Manasarovar, IET 17886 and IET 20443 as tolerant and rest twelve genotypes were susceptible to highly susceptible.

**3.2.5. False smut:** The resistance of rice genotypes to false smut under natural disease incidence was reported by various workers. Out of the seven rice genotypes screened, IRAT 170 was highly resistant, Ex-China was resistant (disease severity score < 1% to false smut), ITA 316 was moderately resistant (disease severity score <5%). Genotype ITA 150 was susceptible, while ITA 315, ITA 335, and FARO 3 were highly susceptible with disease severity scores >20%. The resistance level of Japonica type ranged from 20.37 to 92.90%, whereas, resistance level of Indica rice ranged from 68.15 to 83.21%. Also hybrid rice showed similar Indica rice behaviour whereas their resistance level ranged from 66.82 to 81.88% (El-Shafey 2013).

Seven varieties viz., Ptb 7, Ptb 23, Ptb 24, Ptb 32, Ptb 36, Ptb 42 and Ptb 46 were free from disease when screened under field condition (Raji et al. 2016). Out of 20 varieties screened, the varieties Harsha and Vaishak were found highly resistant and Makom, Thekkancheera, Pavizham and Karthika were resistant and Kanakom, Revathi and Prathyasha showed moderate resistance to the disease (Rashmi et al. 2016). Works at NRRI revealed that, Ranjit and Luna Suvarna, were free from infection. Whereas CR Dhan 907, CR Dhan 303, Nua Kalajeera, Ketakijoha, Nua Dhusara, Nua Chinikamini have exhibited moderate resistance against false smut pathogen.

**3.2.6. Bakanae:** Rice seeds carrying dwarf (d1) and semi-dwarf (d29, sd6 or sdq(t)) genes are useful in resistant breeding program. Recent studies have reported thirteen genotypes with moderate or high resistance, five genotypes with medium resistance, and one genotype with moderate resistance, respectively. Fiyaz et al. (2016) reported eight highly resistant, four resistant, thirty-three moderately resistant genotypes through high throughput screening protocol. Moreover, using inclusive composite interval mapping, three quantitative trait loci, qBK1.1, qBK1.2, and qBK1.3, regulating resistance of rice basmati to *Fusarium fujikuroi* were reported. Two quantitative trait loci (QTLs) on chromosome 1 and 10 were found by an *in vitro* evaluation of the Chunjiang 06/TN1 DH population (Yang et al. 2006). Despite of the necessity of identifying resistance genes and the underlying mechanisms of resistant varieties developed, genetic studies on bakanae disease resistance in rice need to be exploited.

**3.2.7. Sheath rot:** Sixty deep water rice entries were screened to sheath rot disease and reported the cultivars *viz.*, MDR40049, CN 1035-61 and OR-090-3-158 as resistant with less than one percent infection. Of the rest, 32 showed moderate resistant and other 25 showed moderately susceptible reaction. Sahu and Parida (1997) studied the response of 60 rice breeding lines against sheath rot. Out of 60 lines tested, three lines were highly resistant, 18 were resistant and 33 were moderately resistant to the disease. Seed inoculation of rice varieties like BPT-5204, MAS-26 and MAS 946-1 by soaking of seeds in conidial suspension (10<sup>5</sup> condial ml<sup>-1</sup>) of *S. oryzae* overnight was standardized by Mahadevaiah et al. (2016). Twenty aromatic rice genotypes were screened against sheath rot of rice. Less disease incidence were recorded *viz.*, Boga Jalsi, Boga Joha, Monika madhuri Joha, Tulsi Joha, Goul poriya Joha, Bokul Joha



Monipuri Joha, Keteki Joha, and higher disease incidence were recorded *viz.*, Kameni Joha, Badshabhog, Jalsa Joha, Krishna Joha (Singh and Das 2015).

Inspite of the efforts at NRRI for last several years, the availability of resistant genotypes for rice pests at present is very scanty except for BPH, blast and BB (Table 1). Therefore, we should continue evaluating genotypes to obtain resistant donors for other pests, particularly for YSB and sheath blight, which are of economic importance. Further, there is a need to evaluate previously identified genotypes against different pest ecotypes, which may vary in reaction.

Insect/Disease	Source	R/HR* genotype	MR
BPH	NRRI gene bank, breeding	>60	-
	lines of NRRI, IRRI		
WBPH	NRRI gene bank	07	-
Gall Midge	NRRI gene bank,	10	01
Yellow stem borer	NRRI gene bank	-	03
Bacterial blight	NRRI gene bank	43	-
Rice leaf Blast	NRRI gene bank	35	-
Brown spot	NRRI gene bank	-	03
Sheath blight	NRRI gene bank	-	02
False smut	NRRI gene bank	02	06
Tungro	NRRI gene bank	-	09
Angoumois grain moth	NRRI gene bank	-	01

Table 1. Status of resistant genotypes identified against rice pests at NRRI, Cuttack.

## 4. KNOWLEDGE GAPS

Most of the varieties were developed keeping higher yield as the primary criteria and were later found to be resistant/tolerant to pests like BPH. Therefore, in most cases after few years, the resistance break down occurred leading to the havoc of BPH menace. Several resistant donors were identified through screening, but the detail of the mechanism operating for resistance is not worked out properly including molecular basis of resistance. Though several resistance loci have been reported for different pests, most of these are difficult to use during marker-assisted selection due to thorough resolution of some genetic analyses, limited access to donor varieties, and the widespread virulence of insect pests and diseases against certain resistance genes. Resistant donors for most of the necrotrophic pathogens as well as insects such as YSB, leaf folder, case worm and gundhi bug are not yet fully explored. Phenotyping, genotyping, mapping, cloning and characterization of resistance genes against emerging insect pests and diseases are yet to be done. Identification of broad spectrum and durable resistance genes against major insects and disaeses are to be continued. Gene pyramiding to combine major R genes against multiple diseases is to be studied properly along with its impact on crop yield. There is a need to understand the relative importance of both physical and biochemical defense mechanism of plant against insect pests and diseases under induced resistance by different plant elicitors





in rice. This is important for the pests where resistant donors are still lacking. Resistance of the rice varieties (Seeds) to the infestation of stored insect pests is to be exploited for reduction of infestation and also for formulating better storage options for susceptible varieties.

## 5. RESEARCH AND DEVELOPMENT NEEDS

The first and foremost need is to screen a vast number of germplasm against insect pests and diseases under high pressure to identify resistant donors. The mechanism of plant resistance for each identified donor must be understood, particularly against insects through biochemical, antixenosis, antibiosis and tolerance study. Characterization of identified resistant donors for presence of resistance genes using reported markers and identification of new genes, if any. The expression of defense enzymes should be studied in susceptible and resistant lines, particularly, studying the regulation of defense related genes.

The research should be focused to formulate strategies for improving rice pest resistance through genetic studies, plant-pathogen interaction, identification of novel R genes, development of new resistant varieties through marker-assisted breeding for improving rice insect pest and disease resistance in India and worldwide. Genotyping of resistant lines should be attempted by using SSRs, SNPs, and allele mining for major resistance genes. Evaluation of the elicitors must be taken up for induced defense in rice for pest management especially for borer pest and simultaneous identification of genes responsible for defense. There is also a need to test for the effects of foliar deposits of applied silicon on natural enemy foraging and impact. The research should focus on studying the integration cultivar resistance and cultural controls, especially soil silicon amendment, against the entire pest complex in rice. It is necessary to know the resistance/susceptibility of stored rice varieties to the infestation of insect pests, particularly to Sitophilus oryzae and Sitotroga cerealella (Olivier), their occurrence and damage pattern under different storage conditions. Resistant genotypes may be grown in pest endemic areas to assess their performance under different environmental conditions.

## 6. WAY FORWARD

Exploring genetic diversity of rice cultivars for the presence of brown plant hopper (BPH) resistance genes through screening of vast germplasm available at NRRI and National Bureau of Plant Genetic Resource (NBPGR) will pave the way for resistance breeding. At the same time, the valuable resistant genotypes already identified through phenotyping and genotyping should be utilized to develop resistant varieties. They are to be tested also for their reaction against other pests to obtain a multiple resistant donor/variety. Since the susceptibility of rice genotypes to the infestation of stored grain pests depends on the combination of many factors like grain hardness, nutritive value, and natural resistance etc., studies are to be undertaken on these areas for different popular varieties which occupy a greater space in national storage system.

In addition, the factors comprising grain size and moisture content in rice might be the reasons of severe infestation by the rice weevil population. These factors should also be studied along with their management strategies. Important pests and diseases like YSB, leaf folder, gundhi bug, sheath blight, brown spot etc., where resistant genotypes are not available yet, induced resistance work should be in progress for immediate protection of the plant.

The gene/QTL conferring resistance should be identified for further utilization in marker assisted selection of resistant varieties which will quicken the process of varietal development. Although incorporation and utilization of resistance to major rice diseases have succeeded globally, with the currently available biotechnological tools, it is feasible to identify major R genes as well as quantitative trait loci (QTLs) conferring high level of partial resistance and achieve nearly complete resistance against the major and emerging rice diseases.

Identification of donors needs a systematic and continuous evaluation of genotypes for ascertaining their durable reaction. The identified donors can be utilized as genotypes themselves or can be utilized in a breeding programme to develop resistant varieties, may be for a single or for multiple pests. This is the most effective and economic way of pest management system in rice to reduce production cost of farmers as well as pesticide load from the environment.

#### References

- Alam S, Imam J, Nitin M, Prasad C and Variar M (2017) Molecular screening of blast resistance gene Pi2 in Indian rice landraces (*Oryza sativa* L.) and its verification by virulence analysis. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences 87(1): 67-72.
- Bentur JS., Pasalu IC,. Sharma NP, Prasada Rao U and Mishra B (2003) Gall midge resistance in rice. DRR Research paper Series 01/2003, 20pp. Directorate of Rice Research, Hyderabad India.
- El-Shafey RAS (2013) Evaluation of varietal resistance and physiological characters of false and kernel smut diseases of rice in Egypt. Journal of Plant Protection and Pathology. Mansoura University 4(1): 125 – 142.
- Fiyaz RA, Yadav AK, Krishnan SG, Ellur RK, Bashyal BM, Grover N, Bhowmick PK, Nagarajan M, Vinod KK, Singh NK, Prabhu KV and Singh AK (2016) Mapping quantitative trait loci responsible for resistance to bakanae disease in rice. Rice 9: 45.
- Fujita D, Kohli A and Horgan FG (2013) Rice resistance to plant hoppers and leaf hoppers. Critical Reviews in Plant Sciences 32:162-191.
- Goel RK and Bala Ritu (2006) Detection and characterization of resistance to brown leaf spot (*Drechslera oryzae*) in some wild *Oryza* spp. Indian Phytopathology 59: 378 (abstr.)
- Han Y, Lei W, Wen L and Hou M (2015) Silicon-mediated resistance in a susceptible rice variety to the rice leaf folder, *Cnaphalocrocis medinalis* Guenée (Lepidoptera: Pyralidae). PLoS ONE 10(4): e0120557.
- Himabindu K, Suneetha K, Sama VSAK and Bentur JS (2010) A new rice gall midge resistance gene in the breeding line CR57-MR1523, mapping with flanking markers and development of NILs. Euphytica 174:179–187.



- Hossain M, Khalequazzam KM, Mollah MRA, Hussain MA and Rahim MA (2004) Reaction of breeding lines/cultivars of rice against brown spot and blast under field condition. Asian Journal of Plant Science 3: 614-617.
- Hosseini SZ, Jelodar NB and Bagheri N (2012). Study of silicon effects on plant growth and resistance to stem borer in rice. Communications in Soil Science and Plant Analysis 43: 2744 -2751.
- Ikeda R, Khush GS and Tabien RE (1990) A new resistance gene to bacterial blight derived from O.. longistaminata. Jpn J Breed 40 (1):280–28.
- Imam J, Alam S, Variar M and Shukla P (2013) Identification of rice blast resistance gene Pi9 from Indian rice land races with STS marker and its verification by virulence analysis. Proceedings of National Academy of Science India, Section B Biological Sciences 83:499–504.
- Islam F (2007) Factors influencing infestation of rice grains by *Sitophilus oryzae* (Linnaeus) (Coleoptera: Curculionidae). Bangladesh Journal of Zoology 35: 161169.
- Jena M, Panda RS, Sahu RK, Mukherjee AK and Dhua U (2015) Evaluation of rice genotypes for rice brown plant hopper resistance through phenotypic reaction and genotypic analysis. Crop Protection 78: 119 –126.
- Jena Mayabini and Sahu RK (2013) Identification of highly resistant donors and pre-breeding lines against BPH. CRRI Newsletter 34(1): page 15
- Jena Mayabini, Sahu RK and Marandi BC (2006) Screening of rice varieties for resistance against brown plant hopper (Nilaparvata lugens Stal.). Oryza 43(4): 334-335.
- Kalaivani K, Kalaiselvi MM and Senthil-Nathan S (2017) Effect of Methyl Salicylate (MeSA) induced changes in rice plant (*Oryza sativa*) that affect growth and development of the rice leaffolder, *Cnaphalocrocis medinalis*. Physiological and Molecular Plant Pathology. https://doi.org/10.1016/j.pmpp.2017.07.001.
- Katara JL, Sonha H, Deshmukh RK, Chaurasia R and Kotasthane AS (2010) Molecular analysis of QTSs associated with resistance to brown spot in rice (*Oryza sativa* L.). Indian Journal of Genetics and Plant Breeding 70: 17-21.
- Khan HR and Halder PK (2012) Susceptibility of six varieties of rice to the infestation of rice weevil, *Sitophilus oryzae*(L.) (Coleoptera: Curculionidae). Dhaka University Journal of Biological Sciences 21(2): 163-168.
- Krishnamurthy A, Sharma SD and Dhua S R (1995) Miracle rice varieties of India. Central Rice Research Institute, Cuttack.
- Mahadevaiah C, Hittalmani S, Uday G and Prasanna Kumar MK (2015) Standardization of disease screening protocol for sheath rot disease in rice. International Journal of Agricultural Science and Research 5: 129-138.
- Mahender A, Swain DM, Gitishree D, Subudhi HN and Rao GJN (2012) Molecular analysis of native Manipur rice accessions for resistance against blast. African Journal of Biotechnology 11:1321–1329.
- Mohanty SK, Panda RS, Mohapatra SL, Nanda A, Behera L, Jena M, Sahu RK, Sahu SC and Mohapatra T (2017) Identification of novel quantitative trait loci associated with brown planthopper resistance in the rice landrace Salkathi. Euphytica 213:38. DOI 10.1007/ s10681-017-1835-2
- Padmavathi G, Ram T, Ramesh K, Kondala Rao Y, Pasalu IC and Viraktamath BC (2007) Genetics of Whitebacked Planthopper, *Sogatella furcifera* (Horvath), in Rice. Genetics 39(2): 99-105.



- Prahalada GD, Shivakumar N, Lohitashwa H, Sidde Gowda DK, Ramkumar G, Kim SR, Ramachandra C, Hittalamani S, Mohapatra T and Jena KK (2017) Identification and fine mapping of a new gene, *BPH31* conferring resistance to brown plant hopper biotype 4 of India to improve rice, *Oryza sativa* L. Rice 10:41. DOI 10.1186/s12284-017-0178-x.
- Punithavalli M, Muthukrishan NM and Rajkumur MB (2013) Influence of rice genotypes on folding and spinning behavior of leaf folder (*Cnaphalocrocis medinalis*) and its interaction with leaf damage. Rice Science 20(6): 442-450.
- Raji P, Sumiya KV, Dhanya S, Remya K, Narayanankutty MC (2016) Screening of rice varieties and *in vitro* evaluation of botanicals against false smut pathogen, *Ustilaginoidea virens*. International Journal of Agricultural Science and Research 6:79-86.
- Rashmi CR, Gokulapalan C, Raji P, Surendran M (2016) Screening of varieties for resistance to false smut (*Ustilaginoidea virens* (Cooke) Tak. disease of rice. Oryza 53:102-105.
- Sahu AK and Parida S (1997) Screening of new semi-deep water rice selection for reaction to sheath rot and Bacterial leaf blight. Journal of Mycology and Plant Pathology 27: 83-85.
- Satija A, Chahal SS and Pannu PPS (2005) Evaluation of rice genotypes against brown leaf spot disease. Plant Disease Research 20: 163-164.
- Sato H, Ando I, Hirabayashi H, Takeuchi Y, Arase S, Kihara J, Kato H, Imbe T and Nemoto H (2008) QTL analysis of brown spot resistance in rice (*Oryza sativa* L.). Breeding Science 58: 93-96.
- SenthilNathan S, Kalaivani S, Choi MY and Paik CH (2009) Effects of jasmonic acid-induced resistance in rice on the plant brownhopper, *Nilaparvata lugens* Stål (Homoptera: Delphacidae). Pesticide Biochemistry and Physiology 9: 77-84.
- Shafique M and Ahmad M (2003) Susceptibility of milled rice genotypes to Angoumois grain moth, Sitotroga cerealella Oliv. (Lepidoptera: Gelechiidae). SAARC Journal of Agriculture 1: 193-197
- Singh and Das (2015) Screening of aromatic rice (joha) genotype against sheath rot disease of rice and its management under field condition. International Journal of Advanced Research 4: 922-925.
- Stout MJ, Riggio MR and Yang Y (2009) Direct Induced Resistance in Oryza sativa to Spodoptera frugiperda. Environmental Entomology 38(4):1174-1181.
- Vijayalakshmi P, Amudhan S, HimaBindu K, Cheralu C and Bentur JS (2006) A new biotype of the Asian rice gall midge *Orseolia oryzae* (Diptera: Cecidomyiidae) characterized from the Warangal population in Andhra Pradesh, India. International Journal of Tropical. Insect Science 26: 207-211.
- Yadav MK, Aravindan S, Umakanta N, Shubudhi HN, Bag MK, Adak T, MundaS, Samantaray S and Jena M (2017) Use of molecular markers in identification and characterization of resistance to rice blast in India. PLOS ONE 12(4): e0176236.
- Yang CD, Guo LB, Li XM, Ji ZJ, Ma LY and Qian Q (2006) Analysis of QTLs for resistance to rice bakanae disease. Chinese Journal of Rice Science 6: 657–659.
- Yang L, Han Y, Li P, Li F, AliS and Hou M (2017) Silicon amendment is involved in the induction of plant defense responses to a phloem feeder. Science Reports 7: 4232.
- Zhu D, Kang H, Li Z, Liu M, Zhu X, Wang Y (2016) A genome-wide association study of field resistance to *Magnaporthe oryzae* in rice. Rice 9(1): 44.♥



## Bio-ecology of Rice Insect Pests and Diseases: Paving the Way to Climate-smart Rice Protection Technologies

## SD Mohapatra, Raghu S, Prasanthi G, MS Baite, Prabhukarthikeyan SR, MK Yadav, Basana Gowda G, GP Pandi G, A Banerjee, Aravindan S, NB Patil, S Chatterjee, S Lenka, RR Korada, AK Mukherjee, MK Bag, PC Rath and M Jena

### SUMMARY

The insect problem is accentuated in intensive rice cropping where the insects occur throughout the year in overlapping generations. In India, about a dozen of insect species are of major importance in rice but the economic damage caused by these species varies greatly from field to field and from year to year. Insect pests cause about 10-15 per cent yield losses. Estimation states that farmers loose an average of 37% of their rice crop due to insect pests and diseases every year. This chapter focuses on literature generated on various aspects of rice insect pests, diseases *viz.*, pest status and their distribution, bio-ecology, diversity, forecasting model for real-time pest-advisory services, hyper-spectral remote sensing in pest damage assessment, impact of climate change on insect biology, population structure and epidemiology of different rice diseases.

### **1. INTRODUCTION**

The rice plant is an ideal host for large number of insects and pathogens right from nursery to harvest, but only a few of them are considered to be the serious pests those cause economic losses by minimizing the attainable yields. About 800 insect species attack rice starting from their production to consumption and the rest are all friendly insects. Both the mature and immature stages of insect damage rice plants by chewing leaves and root tissues, boring and tunnelling into stems or sucking sap from stems and grains. The injury from feeding leads to damage showing symptoms of skeletonized and defoliated leaves, dead hearts, white ear heads, stunted and wilted plants and unfilled grains. Ultimately insect damage affects the plant physiology leading to reduction in measurable yield, utility or economic return.

In India, about a dozen of insect species are of major importance but the economic damage caused by these species varies greatly from field to field and from year to year. These species include stem borers [yellow stem borer (*Scriphophaga incertulas*), white stem borer (*Scirphophaga innotata*), pink stem borer (*Sesamia inferens*), stripped stem borer (*Chilo polychrysus*), dark-headed stem borer (*Chilo suppressalis*)], leaf folder (*Cnaphalocrocis medinalis* (Guenee), brown planthopper (*Nilaparvata lugens* Stal.). In addition, species distribution and abundance vary among rice

# NRRI

Rice Research for Enhancing Productivity, Profitability and Climate Resilience

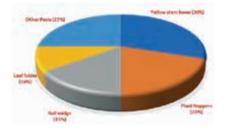


Fig. 1. Estimated yield losses due to different insect pests in rice in India (Modified from http://www.rkmp.co.in ecosystems within a given ecology. For example, termites are primarily upland rice feeders while others are more numerous and damaging under lowland conditions. Some species like yellow stem borer, leaf folder, brown plant hopper may be abundant in all rice-growing environments. Among the biotic stresses insect pests cause about 10-15 per cent yield losses. At National level, stem borers accounted for 30% of the losses, while planthoppers (20%), gall midge (15%), leaf folder (10%) and other pests

(25%) accounted for rest of the losses (Krishnaiah and Varma, 2018) (Fig. 1).

Among the various diseases reported, blast, bacterial blight, sheath blight, stem rot, brown leaf spot and false smut were recorded to be the significant ones. Of these, the sheath blight, bakane and false smut became important only after the green revolution. Some of the diseases appeared in few pockets became major constraints are sheath rot, seedling mortality by *Sclerotium rolfsi*. Primary source of inoculum are internally seed borne, bacterial blight, blast, brown spot, sheath rot (fungal and bacterial), seedling elongation, foot rot, seedling rot by *F. moniliforme*; externally seed borne or as admixture in seed are sheath blight, false smut, seedling blight by *Sclerotium rolfsi*. Some of the soil borne diseases are seedling elongation, foot rot and seedling rot by *F. moniliforme*; sheath blight, false smut, seedling blight by *Sclerotium rolfsi*. Besides the above, all the diseases may spread through collateral host/stubbles of infected plant/diseased plant parts left in the field. Collectively, rice diseases result in yield reductions of 10-15% in tropical Asia.

Crop losses due to arthropods, diseases and weeds across the world have increased from about 34.9% in 1965 to about 42.1% in the late 1990s and the trend is very alarming. Indian farmers face many biotic constraints in their mission to increase rice production. This chapter focuses on literature generated on various aspects of rice insect pests, diseases *viz.*, pest status and their distribution, bio-ecology, diversity, use of hyper-spectral remote sensing in pest damage assessment, impact of climate change on insect biology, population structure and epidemiology of different rice diseases.

Biodiversity conservation has always been major concern and in recent research on this theme has been directed to areas under direct human influence such as agricultural areas where biodiversity performs important functions for productivity through recycling nutrients, regulating the micro-climate and the hydrological processes (Mohapatra 2014). Majority of rice pests are controlled by a complex and rich web of predators and parasitoids that live in or on the rice plant, rice water or soil. The techniques to enhance the biodiversity will enable us to better utilizing the ecological engineering method and opportunities for biodiversity conservation associated with rice fields. There is a need to develop forewarning system, which can





provide advance information for outbreak of insect pests and diseases. Limitation of forewarning model for specific geographic locations could be overcome by the use of satellite-driven weather and agromet data. Resultant systems shall enable appropriate agro-advisory to minimize application of chemical pesticides, losses due to insect pests and diseases, financial burden and environmental cost. Remote sensing gives a synoptic view of the area in a non-destructive and non-invasive way which could be effective and provide timely information on spatial variability of pest damage over a large area. The role of hyperspectral remote sensing in pest surveillance can guide scouting efforts and crop protection advisory in a more precise and effective manner.

A successful pest management plan requires information about a species biology and lifecycle, how it interacts with other species. One pathogen lesion on one leaf does not have a significant economic or ecological impact, however, during an epidemic case even a single lesion leads to causes significant crop loss involves thousands or millions of infections to their host plants. Epidemiology focuses on disease progression, the multiplication of pathogen population through time and the movement of pathogen population from plant to plant. Hence, it is important to understand the population biology and epidemiology of pathogen in order to develop rational control strategies.

To manage the above problems, the present chapter is discussed on the four objectives.

- i. Studies on the faunal diversity *viz.*, insect pests, soil arthropods and natural enemies in different rice ecologies
- ii. Standardization of hyperspectral signature for candidate pests and develop satellite based fore-warning model for major rice pests
- iii. Studies on the population structure of pathogens in different rice ecologies
- iv. Studies on the epidemiology of major and emerging rice diseases

## 1.1. Faunal diversity in rice field

Rice fields together with their contiguous aquatic habitat and dry land comprise changing ecotones, harboring a rich biological diversity, maintained by rapid colonization as well as reproduction and growth of organisms. The variety of organisms inhabiting rice ecosystems includes micro, meso and macro invertebrates (especially arthropods) inhabiting the vegetation, water and soil sub-habitats of the rice fields. In addition, many species of amphibians, reptiles, birds and mammals visit the rice fields for feeding from surrounding areas and are generally considered as temporary or ephemeral inhabitants (Bambaradeniya et al. 1998). In relation to the rice crop, the fauna and flora in rice fields include pests, their natural enemies (predators and parasitoids) and neutral forms.

## 1.2. Lowland rice ecosystem

The rice plant is a host for insects as diverse in their feeding habit as polyphagous grasshoppers and the virtually monophagous white backed plant hopper, *Sogatella* 



Rice Research for Enhancing Productivity, Profitability and Climate Resilience

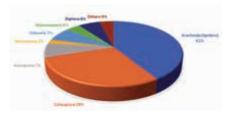


Fig. 2. Abundance of arthropod natural enemies in rice at Upper Gangetic Plain of West Bengal

*furcifera*. Chakraborty et al (2016) profiled the arthropods from the rice field of Upper Gangetic Plain of West Bengal revealed that that herbivores (41%) topped the list followed by predators (21%), parasitoids (16%), detrivours (13%) and plankton feeders (9%). In predatory guild, spiders were dominant group occupied over 41% followed by Coleoptera (29%), Hemiptera (8%), Odonata (8%), Diptera (5%), Hymenoptera (6%) and Neuroptera (2%)

in descending order (Fig. 2). On-farm IPM trial on rice conducted in rainfed low land ecosystem in the Pipili block of Puri district in Odisha revealed that under IPM regime, predator like damsel fly, ground beetle (*Paedarus* sp.), spiders (*Pardosa pseudoannulata, Tetragnatha* sp., *Neoscana theisi*) and mirid bugs were more compared to farmers' practice. The same trend was observed in case of parasitoid complex comprising of *Apanteles* sp. and *Cardiochiles* sp. (Mohapatra et al. 2016). Diversity indices of insect pests and natural enemies differed according to different cultural practices, ecologies and crop growth stages. The highest abundance was at reproductive stage and lowest was at mid tillering stage. They also found that

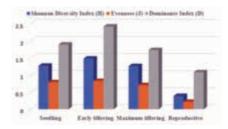


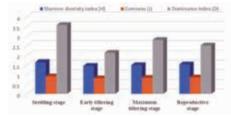
Fig. 3. Diversity indices, evenness and dominance of insect pests at different stages of rice at Bangladesh

transplanted rice fields are richer both in species diversity and species richness. Bakar and Khan (2016) reported that early tillering stage showed higher diversity in terms of diversity index (1.48) compared to other three stages (Fig. 3).

Evenness was also highest in the same stage (0.826) indicating the highest equability among insect pests in that stage. The values of D also differed within different stage and appeared as the highest at early tillering stage (2.42) and lowest at reproductive stage (1.08).

However, 3 diversity indices viz., diversity index (H), evenness (J) and D were found highest at early tillering stage thus seemed to be more stable than other. Similarly, they also reported that the diversity indices viz., diversity index (H), evenness (J) and D of natural enemies in boro rice at different stages were found highest at seedling stage and the lowest at early tillering stage (Fig. 4). Arthropod diversity study undertaken at ICAR-National Rice Research Institute, Cuttack in semi-deep water and irrigated low land ecologies revealed that in semi deep water rice ecology, spiders (7.2/sweep) outnumbered the other predatory groups and were widely distributed throughout the study area. The other major predatory arthropods include damsel fly (5.9/sweep) and lady bird beetle (5.2/sweep).





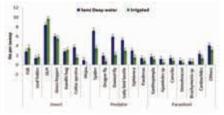
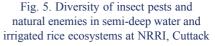


Fig 4. Diversity indices, evenness and dominance of Natural enemies community at different stages of rice at Bangladesh



Among the parasitoids, *Xanthopimpla* sp., *Carcelia* sp., *Stenobracon* sp. *Apanteles flavipes*, *Brachymeria* sp., *Cardiochiles* sp. were the predominant one occurred (Fig 5).

Although the same trends were observed in the irrigated ecology, but the number was comparatively lower than semi deep-water ecology. Diversity indices computed in irrigated ecosystem were Simpson's index [1/D] (10.48), Shannon-Wiener index [H'] (2.62), Margalef's index [M] (2.75) whereas in semi deep water ecologies the Simpson's index [1/D] (13.42), Shannon-Wiener index [H'] (2.78) and Margalef's index [M] (2.76). (NRRI Annual Report 2015-16).

#### 1.3. Coastal rice ecosystem

Coastal rice ecosystem consists of both irrigated uplands and low lands. An experiment conducted at Srikakulam district during *kharif* 2017 depicted that during the first 30 days after transplanting significant yield losses occurred due to BPH, WBPH and leaf folder in low lands of coastal ecosystem. The crop growth period between 30-60 days after transplanting was most vulnerable resulting in major yield losses (20-60%) mainly due to stem borer, leaf folder and brown planthopper. Beyond sixty days after transplanting, the crop damage is inflicted by stem borer and leaf folder causing 10 to 48% damage in coastal ecosystem. The other beneficial fauna prevalent in the coastal ecosystems are coccinellid beetles (5 species), spiders (4 species), earwigs (3 species) and lacewings (2 species). The biocontrol agents include egg parasitioids (2 species) in the coastal ecosystem in north coastal Andhra Pradesh.

## 1.4. Ratoon rice

A ratoon crop is potentially at risk from insect pests because it extends the time period of host availability. With its shortened vegetative stage, a ratoon crop is unsuitable to early season insects like whorl maggot, *Hydrellia philippina* and caseworm, *Nymphula depunctalis*. Stem borer numbers are significantly reduced at main crop harvest, but some survives to attack the ratoon crop. Leaf folder infestation is higher at the vegetative stage on a ratoon crop than main crop. The lack of land preparation in establishing a ratoon crop allows a high carryover of natural enemies, particularly the predators which prevent significant build up of brown planthopper, white backed planthopper and green leafhopper.



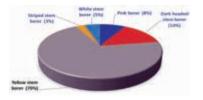


Fig. 6. Relative abudance of different species of stem borer

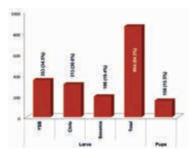


Fig. 7. Abundance of rice stem borer in stubbles in ICAR-NRRI, Cuttack

The incidences of diapausing rice stem borer larvae in the rice crop residues (stalks and stubbles) investigated at ICAR-NRRI, Cuttack from December 2016 to March 2017 revealed that three predominant stem borer larvae diapaused in the rice crop residues were Yellow stem borer, Scirpophaga incertulus, Striped stem borer, Chilo supressalis and Pink stem borer, Sesamia inference. The relative abundance of three diapausing stem borers revealed that yellow stem borer (40.8%) was most predominant followed by Striped stem borer (36.2%) and Pink stem borer (23%). The occurrence of stem borer species was correlated with the height of the stubble. The results revealed that 60% of the total S. inferens and 17.6% of the total Chilo sp recorded were concentrated to 9.8 and 7.0 cm above the root zone, respectively, whereas 98% of the Yellow stem borer larvae were concentrated to the base of rice stubbles (Fig. 6 & 7).

It is clear that destruction of diapausing larvae of the above species of rice stem borers during land preparation would be an effective cultural control method (NRRI Annual Report 2015-16). During crop harvest, if rice stubble heights are adjusted to a range of 5–10 cm, the survived stem borers can be reduced by 70–90%. Furthermore, approximately 70% of the overwintering stem borers can be killed by ploughing and irrigation. These measures can significantly decrease the initial population sizes of stem borers.

#### 1.5. Natural enemies

In agro-ecosystems, the associated natural enemies can perform important ecological services, mainly biological control of crop pests. Naturally occurring biological control has a potential role to play in the management of rice fields of tropical south and south-east Asia and there is a need to emphasize the impact of indigenous natural enemies as an essential part of IPM programme. Conservation of the natural enemy fauna in situ for suppressing the pest population seems to be a very good alternative. A study conducted at Cauvery command areas of Karnataka by Parasappa et al. (2017) indicated that among the various predators, spiders and mirids were the most important natural enemies. Among the odonata, damselflies population was more compared to the dragonflies. Mirids *Cyrtorhinus lividipennis* was considered as important, potential and efficient predator of BPH and WBPH. Staphylinids were identified as *Paederus fuscipes* which is a predator on leafhoppers. Rice insect predators in India belong to 25 families of 6 orders of class Insecta. Among the predacious orders, Coleoptera ranks first and the family Coccinelidae is





exclusively predacious with a few exceptions. Predaceous carabid beetles are generally recognized as useful natural enemies against lepidopterous larvae in the rice fields. There are 368 coleopteran species associated with rice and there is always confusion about their herbivorous or beneficial role. Hymenopterous parasitoids associated with rice are 524 species belonging to 181 genera exercising natural control. Among the 419 organisms enumerated during the investigation, hymenopterans were by far the most abundant representing 191 species followed by coleopterans and dipterans representing 38 species each.

Predators are the most conspicuous and consume many preys during their life span. Dragon flies and damsel flies are amongst the most conspicuous insects associated with irrigated rice fields. They predate on adults of yellow stem borer and leaf folders. Fourteen spp. of dragonflies and damselflies have been recorded from rice field at Cuttack.

**1.5.1. Spider:** Spiders such as the wolf spider, Lynx spider, Orb spider are known to consume a large number of prey and play an important role in reducing the densities of plant and leaf hoppers in rice fields. Spiders are thus known as biological control agents for phytophagous insects with some species being able to reduce the total pest population by 22% per day. The predominant species of predatory spiders were reported from rice fields of North–Eastern UP were *Tetragnatha javana*; *Pardosa pseudoannulata*; *Tetragnatha mandibulata*, *Pardosa birmanica*, *Hippasa holmerae*, *Tetragnatha maxillosa*,

**1.5.2. Bird:** In rice cultivation, many farmers have the practice of keeping dried tree stumps in different localities in the field. Birds such as Drongos, Mynas and Kingfishers use perches to feed on insects and caterpillars from the rice fields during daytime. At night, different species of owls use these perches to prey upon rodents that contribute to crop loss. Another group of birds that play a vital role in rice fields is the Egrets, Herons and Water hen. These birds feed on worms, moths and caterpillars as well as harmful soil organisms. Flock of cattle egret, *Bubulcus ibis* running behind the plough or country plough is a very common sight in the rice paddy.

**1.5.3. Earthworm:** Earthworms are the most important soil dwelling organisms involved in the process of soil formation and maintenance of soil health and help the composting process. Earlier ten different earthworm species have been identified from different habitats belonging to 4 different families. *Lampito mauritii* and *Pellogaster bengalensis* were found to be ubiquitous. Among them, only *L. maurtii* was found in all sites in high numbers

**1.5.4. Duck:** Ducks are generalist predators, feeding on stem borers, leaffolders, grasshoppers, planthoppers and leafhoppers etc. Ducks have big appetite and on an average one duck can consume more than 100 insects per hour thus decreasing pest populations quickly, particularly in the early to mid-tillering stage of rice. The total number of plant hoppers and leafhopper in duck fields were reduced by 63.9 and 77.3%. Rice–duck farming agro-ecosystem reduced 30.6% fertilizers and 59.4% pesticides usage compare with conventional farming system.



**1.5.5. Fish:** Fishes like carp, tilapia and catfish feed on plant and leaf hoppers, stem borers or other insects like mosquito larvae and other aquatic insects that fall into the water. Some fish also feed on the outer leaf of the leaf sheath, which contains plant hopper and leaf hopper eggs.

**1.5.6. Soil arthropod:** Soil mesofauna which includes Cryptostigmatids, soil Acari including Oriebatid and Cryptosigmatid mites and other invertebrates as important reservoir of biodiversity and plays a pivotal role in determining many soil characteristics. This diverse group of animals cover a range of taxa, the most important being protozoan's, nematodes, oligochaete worms (earthworms and enchytraeids), mites, collembolans, millipedes, centipedes and a range of insects whose larval stages complete their development in the soil. The presence of soil fauna increased the soil organic carbon and also helps in decomposition of organic matter which led to an increase in the yield of the crop by increasing the availability of nutrient. Some of soil arthropods like ants and termites increased soil water infiltration due to their tunnels and improved the soil nitrogen recycling of crop residues and plant productivity and keeping the balance of soil carbon pool as well.

## 2. FORECASTING MODEL FOR MAJOR INSECT PEST AND DISEASE

Weather-based forecasting systems reduce the cost of production by optimizing the timing and frequency of application of control measures for minimizing crop loss and reducing cost of plant protection. Forecast models provide an alternative to calendar spray schedule to bring need-based protection, e.g., instead of sprays at 7-14-day intervals to spray at precise time just when and where the pest is likely to appear. Thus, precision pest management may bring down number of chemical pesticide sprays to provide economic and environmental benefits. Finally, the system of forecast should be taken as economically acceptable action as an integral part of IPM package while growers should be capable and flexible enough to take due advantage of a pest forewarning advisory.

## 2.1. Stem borer

Rice yellow stem borer, *Scirpophaga incertulas* recorded were used in conjunction with the weather data of a particular location for development of weather-based prediction categorized as to low, medium and high severity. The weather-based criteria and prediction rules have been integrated online for forewarning *S. incertulas* population levels. Forewarning of *S. incertulas* is specific for Cuttack location for *kharif* season and being used for pest advisory services for the rice growers of the region.

## 2.2 Rice blast

Savary et al. (2012) in Korea developed EPIRICE, a generic model for plant diseases which was coupled with GIS. Manibhushanrao and Krishnan (1991) developed EPIBLA (EPIdemiology of BLAst) model using multiple regression equation based on maximum





temperature and maximum RH for simulation of leaf blast incidence. In their model simulated incidence of blast made 7-day forecasts of disease progression in many parts of India. The ICAR- NRRI, Cuttack operated a simple leaf blast forecasting system based on empirical factors which interacted with rice varieties. Stages which are most susceptible to rice blast viz. seedling, tillering and flowering were identified. Kaundal et al. (2006) introduced a machine learning techniques model for forecasting rice blast in India. Six weather variables were selected viz. temperature (max and min), RH (morning & evening), rainfall and rainy days per week. Blast is mostly preferred by particular air and soil temperatures, relative humidity (RH), hours of continuous leaf wetness (LW), degree of light intensity, duration and timing of dark periods. All of these have been considered as very crucial for development of the disease.

# 3. HYPER SPECTRAL REMOTE SENSING FOR PEST SURVEILLANCE

At present, satellite remote sensing data are also being used in generating and improving weather forecasts, providing crop estimate in terms of net sown area and yield, issued in operational mode for the last few years with reasonable accuracy for rice, wheat, mustard, potato, etc. Use of Remote Sensing (RS) and Geographic Information System (GIS) could be explored for analysis of satellite-based agro-met data products, mapping geographical distribution of pests and delineating the hotspot zones. Super-imposition with causative abiotic and biotic factors on visual pest maps can be useful for pest forecasting. Since, damaged plants increase reflectance particularly in chlorophyll absorption band (0.5-0.7 cm) and water absorption bands (1.45-1.95 cm), forecasting crop pests is possible by remote sensing.

## 4. CLIMATE CHANGE AND RICE INSECT PESTS

Current estimates of changes in climate indicate an increase in global mean annual temperatures of 1 °C by 2025 and 3 °C by the end of the next century. The date at which an equivalent doubling of  $CO_2$  will be attained is estimated to be between 2025 and 2070, depending on the level of greenhouse gases emission. Fifteen studies of crop plants showed consistent decreases in tissue nitrogen in high  $CO_2$  treatments; the decreases were as much as 30%. This reduction in tissue quality resulted in increased feeding damage by pest species by as much as 80% (Coviella and Trumble 1999). In general, leaf chewers (Lepidoptera) tend to perform poorly whereas suckers (aphids) tend to show large population increases indicating that pest outbreaks may be less severe for some species but worse for others under high  $CO_2$ .

Natural enemy and host insect populations may respond differently to the global warming. There also instances where warmer conditions increase the effectiveness of many natural enemy species and/or increase the vulnerability of their prey. In extreme conditions, higher abundance of insect pests may partly be due to lower activity of parasitoids or to disturbed parasitoid-pest relationship and decreased controlling ability. However, parasitoids [*Anagrus incarnatus* and *Apanteles chilonis*] and



predators (*C. lividipennis*), other than spiders (*Pardosa astrigera* and *Tetragnatha vermiformis*) of rice insects breed two to three more generations a year. These facts imply that the extent of biological control of rice pests by natural enemies will increase in intensity under the global warming.

## 4.1 Carbon dioxide

The majority of plants, particularly those in the C<sub>3</sub> category, which includes rice, respond to increased CO, levels by increasing productivity in the form of carbon fixation. A CO<sub>2</sub> induced reduction in host plant quality resulted in increased larval consumption rates in order to obtain adequate dietary nitrogen in generalist. In the majority of cases, increased feeding rates do not compensate fully for the reduced quality of the diet, resulting in poor performance, slowing insect development and increasing length of life stages which place them vulnerable to the attack by parasitoids. However, the change in C:N ratio in the plant, phloem sap becomes more concentrated at higher temperatures, and thus acts as a richer source of amino acids for sap feeders. The concentration of a range of secondary plant compounds tends to increase under drought stress, leading to changes in the attraction of plants to insects. The atmospheric environment in the future is predicted to include correlated increases in CO<sub>2</sub> concentration and temperature. While crop biomass is predicted to increase in response to elevated CO<sub>2</sub> concentrations under many circumstances, it is also recognized that crops and soils may subsequently become nutrient limited, especially in terms of nitrogen availability.

## 4.2 Temperature

Insects are ecto-thermic organisms, the temperature of their body changes approximately with the temperature of their habitats. Therefore, temperature is probably the most important environmental factor influencing their behaviour, distribution, development, survival and reproduction.

## 4.2.1 Yellow stem borer

Yellow stem borer took 8.1 mean days for hatching out into larvae at 30 °C. However at higher temperature hatching period was found to be decreased. In the same way, larval and pupal developmental period changes with increasing temperature. The percentage incidence of dead heart and white ear heads were correlated negatively with rainfall and minimum temperature, and positively with maximum temperature. The percentage of white ear head correlated negatively with relative humidity. Manikandan et al. (2013) reported that the number of eggs laid by YSB increased at higher temperature. At 28.3 °C, the YSB laid 143 eggs, whreas it was increased to 176.5 eggs at 36 °C with a standard deviation of 6.6. Insect populations from environments with higher temperatures may have higher fecundity and shorter growth stage. It is reported that the incubation period of Scirpophaga incertulas decreases at higher temperature, beginning at 30 °C and continuing up to 35 °C. Egg hatching percentage of the YSB decreased at higher temperature and increased at lower temperature. The egg hatching percentage was high (90.6%) at 30.6 °C followed by 28.3 °C. In contrast, only 58.5 per cent of incubated eggs achieved emergence at 36.0 °C. The incubation period of YSB eggs was 8.5 days at 28.3 °C, whereas it took only 5.75 days at 36 °C.



The development time taken by the four larval instars varied significantly with respect to the temperature.

#### 4.2.2 Brown plant hopper

Brown plant hopper requires 6.7 mean days for hatching out into nymphs in ambient condition. However, this period decreased significantly at higher temperature. Decreased developmental duration of instars observed at increasing temperatures might be connected with faster larval growth at these temperatures. Insects develops faster will oviposit early and hence the population will grow earlier than expected. The total life span at 38 °C decreased significantly than at 30 °C.

#### 4.2.3 Leaf folder

Drastic changes in temperature can cause oxidative stress, which in turn trigger the production of reactive oxygen species (ROS) derived from the metabolism of molecular oxygen which cause oxidative harm to proteins, nucleic acids, lipids. Insects produce a number of antioxidant enzymes for detoxifying ROS. Oxidative stress enzymes viz., glutathione s-transferases (GSTs), catalase (CAT), superoxide dismutase (SOD) play an inevitable role in detoxifying mechanism of ROS and contribute to regaining the balance.

One response to warm stress is the formation of reactive oxygen species (ROS) causing oxidative harm. The raised levels of SOD and GSTs movement, shown in a period played an important role in battling of ROS in *Neoseiulus cucumeris* demonstrated the contribution of these enzymes in host protection against thermal stress. The ability of an insect without compromising the pace of its growth and development to tolerate the thermal stress is an important adaptation to survive in various climatic conditions (tropical, subtropical, and temperate), which is vital in predicting insect outbreaks.

#### 4.2.4 Stored grain pests

The stored grains maintained at a sufficiently low moisture level can be stored for many years without any significant loss in quality. Optimum grain moisture for development and reproduction of insects is 12.0 to 14.0 per cent. Generally, the dormant stages-eggs and pupae of insects, eggs and resting stages of mites, and spores of fungi can best resist desiccation while acting feeding stages may die out if conditions are too dry.

Angoumois grain moth is one of the most serious pests of stored rice (paddy) at post-harvest level. Three temperature zones are significant for growth and death of stored product insects. At optimal temperatures (25-32 °C), insects have maximum rate of multiplication. At sub optimal temperature (13-24 °C and 33-35 °C) where development slows, and at lethal temperatures (below 13 °C and above 35 °C) triggered the insects to stop feeding, develop slower, and eventually die. The more extreme temperature, the more quickly they die. Each insect species, stage and physiological state will affect the particular response to temperature. No stored-product insects can survive freezing.



Stored product insects breed faster at high humidity (65-80%) which is approximately equal to 13.0 - 15.0 per cent moisture content of the grains. Above 80 per cent humidity or 15 per cent moisture content, mould growth start suppressing insect multiplication. Rice weevils complete their life cycle in 25 days at 30 °C and while they take about 94 days at 18 °C. At a temperature over 34 °C, insects usually cannot develop. However, lesser grain borer *Rhyzopertha dominica* has the shortest development period of 25 days at 36 °C with 80.0 per cent relative humidity and longest development period of 106 days at 20 °C with 60.0 per cent relative humidity. At 20 °C developmental activities of larvae and pupae of *Tribolium castaneum* ceased and at 35 °C, it retarded significantly. The highest population increase of *S. cerealella* occurred at 30 °C.

## 4.3 Elevated temperature and carbon dioxide

Earlier studies predicted that elevated CO<sub>2</sub> and temperature exhibited a significant positive effect on BPH multiplication and its population than ambient CO, and temperature (Pandi et al. 2016). Rice plants exposed to elevated conditions recorded higher number of eggs  $(303.2 \pm 35 \text{ eggs/female})$  whereas in the plants under ambient condition  $(212.9 \pm 21.5 \text{ eggs/ female})$  female laid significantly less number of eggs. Thus, it was revealed that elevated condition stimulated fecundity of BPH by 29.5 % compared to ambient. Quantification of honeydew was directly related to the sucking rate; where only under elevated CO, condition honeydew excretion was significantly higher than ambient condition. In contrast elevated CO<sub>2</sub> and temperature honeydew excretion did not differ significantly from ambient condition. Further, developmental period of nymphs and longevity of brachypterous females were significantly reduced under elevated condition as compared to ambient. It has been observed earlier that every degree rises in global temperature, the life cycle of insect would be shorter. The quicker the life cycle, the higher will be the population of pests. Combined effects of both elevated temperature and CO<sub>2</sub> altered the plant phenology and pest biology and aggravated the damage by brown planthoppr (BPH), Nilaparvata lugens.

## 4.4. Precipitation

Many pest species favour the warm and humid environment. Both direct and indirect effects of moisture stress on crops make them more vulnerable to be damaged by pests, especially in the early stages of plant growth. Some insects are sensitive to precipitation and get killed or removed from crops by heavy rains. A decrease in winter rainfall resulted in reduced aphid developmental rates because drought-stressed tillering cereals reduce the reproductive capacity of overwintering aphids.

## 5. POPULATION BIOLOGY OF RICE PATHOGENS

## 5.1. Rice blast

*Magnaporthe oryzae* (63 isolates) collected, of which 16 (25%) were the mating type MAT1-1 while 35 (56 %) were mating type MAT1-2. The MAT1-2 isolates predominated in Jharkhand and Assam while MAT1-1 is more predominant in the



isolates of Odisha. Both MAT1-1 and MAT1-2 were equally distributed in the isolates of Meghalaya and Tripura. In another study forty six isolates of *M. oryzae* were collected from various ecosystems of coastal Odisha, and the mating type analysis showed that MAT1-1 mating type was dominating in all the ecosystems and MAT1-2 was found to be present in uplands as well as in irrigated fields. Both mating types could be found in the same field in irrigated ecosystem.

Recently, twenty isolates of *M. oryzae* were collected from Chhattisgarh and categorized into three groups based on colony colour *i.e.*, greyish blackish, greyish and white, and in two group based on the texture of the colony as smooth and rough. All the twenty isolates produced the characteristics symptoms of spindle shaped lesion on susceptible plant. Among them, 5 isolates were found to be highly virulent, 8 were moderately virulent while, 7 were mild in nature. In phylogenetic analysis, overall two major groups were formed. The Chhattisgarh (CG-2 and CG-43) blast isolates along with Indian isolate were in one group whereas; isolates from Brazil, Kenya, Japan and China were in a separate group.

## 5.2. Sheath blight

Sheath blight (ShB) of rice caused by *Rhizoctonia solani* Kuhn [teleomorph: *Thanatephorus cucumeris* (Frank) Donk] is a major biotic constraint of rice in almost all the rice growing tracts of India. Yield losses due to this disease were estimated to range from 1.2 to 69.0% depending on the cultivar, environmental condition and crop stage. The pathogen has a wide host range and can infect plants belonging to more than 32 families and 188 genera. The weeds in and around the rice fields, water channel and irrigation ponds may serve as source of primary inoculum of the fungus. Natural occurrence of *Rhizoctonia solani* has been reported on sugarcane, weeds, wheat, bajra, cash crops such as cotton, coriander, and turmeric. Sheath blight pathogen survives from one crop season to another through sclerotia and mycelia in the plant debris and also through weed hosts in tropical environments. Both mycelia and sclerotia survive in infected plant debris. The disease severity was positively correlated with sandiness of soil. Further, the disease incidence was highest in wet soils with 50-60% water holding capacity (WHC) and lowest in submerged soils with 100% WHC.

The extent of damage of rice seedlings due to sheath blight incidence is dependent on resistance levels among the rice strains, average daily temperature and frequency of rain. Pot culture studies on the susceptibility of rice seedlings to *R. solani* revealed that disease incidence and development was rampant on 20 to 30 days-old rice seedlings compared to seedlings of 30 to 40 days old under artificially inoculated conditions. Rice ShB symptom production under artificial condition depends on the method of inoculation. Of different inoculation techniques such as single grain insertion, single sclerotium insertion and mycelial suspension injection; single sclerotium insertion was found most effective with highest ShB symptoms (68.5 to 80.0%), lesion length (2.45 to 4.75 cm) and percent disease index (32.5-43.5) followed by single grain insertion technique.

Maximum disease severity was observed when sheaths and leaves were inoculated with 7-day-old propagules of the pathogen. The amount of R. solani inoculum plays a major role in ShB disease development. Inoculum at the rate of 0.2 mg when placed inside the leaf sheath with a few drops of sterile water induced single, discrete and uniform-sized lesions irrespective of the inoculum type (mature, immature sclerotium, and mycelium). Early infection on a healthy plant within 12 h is possible when mycelium of the pathogen was used instead of sclerotial bodies. The ShB pathogen can infect the rice crop at any stage of growth from seedling to flowering by different inoculum sources. Three pathogens are found to cause ShB disease in rice. They are R. solani (Thanatephorus cucumeris), R. oryzae-sativae (Ceratobasidium oryzae-sativae) and R. oryzae (Waitea circinata). Combined inoculation with these pathogens resulted in highest disease severity. Further, ShB incidence was maximum when treated with R. solani, moderate with R. oryzae-sativae, and low with R. oryzae. Multiple linear regression test was made between the percent disease incidence (PDI) and the weather parameters indicating the highest contribution (61.05%) came from rate of evaporation, while the other two weather parameters viz., maximum and minimum temperature contributed 9.03% and 23.03% respectively.

## 5.3. Bacterial blight

The monitoring of pathotype is still an important tool for providing timely information about the population structure of the pathogen and the effect of climate change on population structure. In a recent study Yugander et al. (2017) reported that bacterial blight pathogen, *Xanthomonas oryzae pv oryzae* has invaded into the newer areas with more virulence. In fact the evolution of new races in plant pathogen is a continuous process which requires regular monitoring. The change of climate especially the increase of temperature with high humidity has helped the *X. oryzae pv oryzae* to gain more virulence and that's why newer areas were also invaded by this pathogen. It is interesting to observe that different researchers have reported presence of different pathotypes of X. *oryzae pv oryzae* in India (Nayak et al, 2008; Yugander et al. 2017) for further study on the pathogen population infecting rice with the help of differentials and molecular markers available.

## 5.4. Sheath rot

The rice sheath rot has gained the status of a major disease of rice and yield loss varies from 3 to 85%. Rice sheath rot is a disease complex that can be caused by various fungal and bacterial pathogens. Major pathogens associated with rice sheath rot disease are fungi such as *Sarocladium oryzae* and *Fusarium* sp. belonging to the *Fusarium fujikuroi* complex and the bacterial pathogen *Pseudomonas fuscovaginae* (Bigirimana et al. 2015). *S. oryzae* is present in all rice-growing countries worldwide, being very common in rainy seasons. The pathogen survives in infected seeds, plant residues (straw and stubble), but also in soil, water or weeds when environmental conditions are favorable. Helvolic acid and cerulenin are described as the major secondary metabolites of *S. oryzae* and the pathogenicity determinant of the disease. Temperature of 20-30 °C and relative humidity of 65-85% favour the sheath rot development.



Sheath rot in rice has also been associated with *Fusarium* sp. belonging to the *F. fujikuroi* complex. The complex is currently divided in three large clades, the African clade, the Asian clade and the American clade. The main organisms associated with rice are *F. verticillioides* from the African clade and the closely related species *F. proliferatum* and *F. fujikuroi* from the Asian clade. Symptoms of rice sheath rot caused by any of the members of the *F. fujikuroi* species complex are wide spread due to their large variability and at least one of their members is found in any part of the rice-growing world. Two categories of metabolites are involved in pathogenicity and interaction with plants, gibberellins and mycotoxins. *F. fujikuroi* can survive up to 26 months in infected rice grains. Rice sheath rot causing *Fusarium* spp. have many hosts, they can easily find alternate hosts in the environment, especially weeds.

## 5.5. False smut

Rice false smut caused by the fungal pathogen *Ustilaginoidea virens* (Uv) is becoming a destructive disease throughout major rice-growing country. Information about genetic diversity and population structure of the pathogen is essential for rice breeding and efficient control of the disease. Recent reports applying genomic and transcriptomic data have revealed single nucleotide polymorphism (SNP) and simple sequence repeat (SSR) markers for the identification of Uv genetic diversity (Sun et al. 2013). Earlier studies using PCR-based approaches, such as rDNA-ITS variability amplified fragment length polymorphism (AFLP) and random amplification of polymorphic DNA (RAPD), have identified very limited genetic diversity of Uv.

However, three SNP-rich genomic regions have been identified by comparative genomics. Based on the analysis of the three SNP-rich genomic regions, significant genetic diversifications were detected among populations from five major rice production areas in China, and isolates from the same area showed considerable DNA composition stability, which consistent with the conjecture that Uv may not be an air-borne, but a water- and/or soil-borne pathogen. Consistent with this speculation geography is more important than rice cultivar in constructing the genetic diversity of Uv. Interestingly, genetic divergence is generally higher in isolates from inland areas than from coastal areas. Genetic variation in north-east China is relatively low, which may be a result of less active sexual reproduction. Survey of literatures reveals that except Baite et al (2014) who reported that the genetic variability of Indian isolates was related to geographical location as isolates from distantly related locations possess higher genetic diversity; there is no other reports on population structure of UV which is a good researchable issue that may be taken up in future.

## 5.6. Bakanae

In India, bakanae disease is also called as foolish seedling or foot rot because of the variable symptoms, the pathogen produces. The disease is monocyclic with pathogen producing conidia on infected plants and conidia will spread by wind and water. The high production of conidia on infected or dead culms in the field coincides with flowering and ripening of rice, when the conidia are able to infect or contaminate



the seeds (Infected kernels develop a reddish color due to the presence of conidia, and the whole seed becomes discolored when severely infected). The fungus can also be isolated from asymptomatic seeds, if they are collected from a highly infected rice field. Airborne ascospores have also been reported, as an infection source, at the flowering stage of the crop. The fungus can infect seedlings at an early stage of development, when it becomes systemic in the plant, but without any colonization of the floral organs. The first 72 hours after seed germination are critical for the development of the disease, which is favoured by high amounts of exudates (sugars and amino acids) from germinating seeds.*F. fujikuroi* growth is also stimulated by temperatures from 27 °C to 30 °C, and by higher levels of nitrogen in the soil.

Soil temperature also plays a crucial role in disease development, with more prominent bakanae symptoms at 35 °C soil temperature. The application of nitrogen to the soil stimulated the development of the disease and the effect was not modified by the application of potassium or phosphorous. Relative humidity also plays an important role in disease development with high humidity leads to elongation of the culms, while low humidity causes rice plant stunting (Matic et al. 2016). Microconidia and mycelia of the pathogen develop in vascular bundles, particularly in larger vessels and in the xylem gaps, while the phloem and parenchyma do not seem to be infected. The fungus overwinters in infected seeds, and these represent the main source of inoculum for the following season. Progress in molecular taxonomy has shown that there are around 50 species in the *F. fujikuroi* complex and the number keeps increasing. The complex is currently divided in three large clades, the African clade, the Asian clade and the American clade. The main organisms associated with rice are *F. verticillioides* from the African clade and the related species *F. proliferatum* and *F. fujikuroi* from the Asian clade.

## 6. EPIDEMIOLOGY

## 6.1. False smut

Epidemiological study of false smut pathogen is essential to gather information for formulating appropriate management options. Till date there is no definitive pattern of infection process, dissemination method and the influence of weather factor vis-àvis combination factors responsible for severe infection of false smut pathogen to rice. Nessa et al. (2015) provided a broad but relatively clear picture of on the epidemiology of rice false smut disease under natural environment and reported that soil is the source of initiation of epidemic but did not recognize any long or short distance primary or secondary source of infection. At Temperature 22-25 °C with no less than 48 h of wetness duration considered necessary for successful infection of sexual stage of FS pathogen *Villosiclava virens* and the highest level of disease (92.9%) was obtained at 25 °C and 95% RH with 120 h wetness. Light can inhibit the formation of secondary spores from chlamydospores. High level of nitrogen fertilization increases rice foliar growth which allowed for higher humidity below the canopy and created an environment favourable for the development of RFS. Additionally, irrigation has been found to be a major factor which affects the development of RFS. Lower



minimum and maximum temperature, high atmospheric humidity (92% and above) before and during early part and less during later part of flowering favoured the disease.

## 7. DIAGNOSTIC ASSAY

## 7.1. Rice blast

Highly sensitive and accurate methods for the early diagnosis of *M. oryzae* will reduce quantum of loss. Traditionally, the major technique used to detect plant pathogens is based on cultural and morphological observation. Consecutively, some immunoassays and nucleic acid-based techniques have been developed for the diagnoses of plant pathogens. The enzyme-linked immunoasorbent assay is a sensitive and specific method for the detection of plant pathogenic fungi. The most important is the polymerase chain reaction (PCR) based detection methods that are more accurate, sensitive and specific for diagnosis of blast disease. The quantification of M. oryzae growth in rice plant was developed based on RNA-based northern blotting and DNA-based real-time PCR.

#### 7.2. Storage pathogen

Rice suffers from more than 60 diseases and most of the major diseases of rice are seed borne. Fungi are the principal organisms associated with seed storage (Fakir 2000). Bacteria are also commonly carried internally or externally by the seeds. The extremely seed borne diseases of rice are brown spot, bakanae, blast, sheath blight, sheath rot, stem rot, false smut are major seed borne pathogens of rice. Bacterial pathogens such as bacterial leaf blight and bacterial leaf streak are also taking a heavy toll in terms of yield and quality by rapid spread and damage. Seed may be infested, contaminated or infected. Seed infection may take place through the mother plant, invasion through natural openings including the functes and microphyles, direct penetration of the seed or caryopsis or invasion from the pods or fruits.

Conventional detection and diagnosis methods coupled with molecular techniques can add to the rapid and accurate diagnosis. In rice, a BIOPCR technique was used to study survival of *Xanthomonas oryzae* pv. *oryza* in rice seed and track its progress in planta following seed transmission. Quantitative real time PCR detection will definitely help to quantify the pathogen load at an early stage so that further losses can be minimized. Loop-mediated isothermal amplification (LAMP) offers as a field oriented and user-friendly alternative to polymerase chain reaction (PCR). LAMP is less time intensive than PCR and can be performed using heat-blocks, with results read by eye under UV light.

# 8. KNOWLEDGE GAPS AND RESEARCH AND DEVELOPMENT NEEDS

The biotic stresses are major contributors to reduction of crop yield. Increasing ozone, CO, and greenhouse gases in the farming atmosphere are possible reasons for



increasing pest pressure and change in pest species to rice crop. Scientific tools and techniques would ease the stresses endured by pest and diseases and shall provide better initiatives amongst entrepreneurial initiative.

Biotic menaces are weather-dependent, weather-based prediction models could be developed to manage these menaces. Forewarning models for major pests of rice using satellite-based agromet product and surface data could be developed for decision support system(s), which would reduce use of chemical pesticide on standing crop. Besides, the measurement of insect developmental, survival and reproductive responses to temperature poses practical challenges because of their modality, variability among individuals and high mortality near the lower and upper threshold temperatures. It will aid in the development of powerful tools for analyzing insect population behaviour and response to challenging climatic conditions. It is important to quantify and verify by critical experiments, the speculative relationships frequently proposed between climatic factors and the population dynamics of rice insects. Future experiments with population in controlled environments as well as statistical correlations based on field data will permit a much clear understanding of the importance of climate, and reveal the potential for improving pest control methodology through this understanding

## 9. WAY FORWARD

With the available scientific knowledge, there is need for further strengthening the research and development in the following areas:

- How temperature and other abiotic factors set the limits of distribution and define abundance of insect species.
- The physical and biological components of our environment are all interrelated. Thus, the rice fields need to be given the attention they need and deserve. Preventing alien species from invading the rice ecosystem is very important. Alien species often affect the conservation of endangered species by competition and inducing additional chemical control applications.
- The importance and abundance of natural enemies have not previously been investigated in different rice ecologies like upland, lowland, irrigated and deep water. The current work will address the paucity of information on enemies of paddy pests thriving in different rice ecologies and to have their comparative diversity.
- Issues and problems about rice fields should be taught in schools. Students should understand what is happening to a vital ecosystem such as rice fields so that they could make a stand and help preserve an important part of our environment and economy. Rice fields offer many benefits for all of us, like better rice and more food and better environmental safety.



## **10. CONCLUSION**

The success of rice disease and pest management involves the understanding of various aspects of rice insect pests, pathogens, their pest status, distribution, bioecology, diversity, forecasting model for real-time pest-advisory services, hyperspectral remote sensing in pest damage assessment, impact of climate change on insect biology, population structure and epidemiology of different rice pathogens. These knowledge will reduce chemical pesticide application in rice, financial burden and ultimately reduce environmental pollution.

#### References

- Baite MS, Sharma RK, Devi TP, Sharma P and Kamil D (2014) Morphological and molecular characterization of *Ustilaginoidea virens* isolates causing false smut of rice in India. Indian Phytopathology 67(3):222-227.
- Bakar MA and Khan MMH (2016) Diversity of insect pests and natural enemies as influenced by growth stages and pest management practices in rice. Bangladesh Journal of Agricultural Research 41(3):461-470.
- Bambaradeniya CNB, Fonseka KT and Ambagahawatte (1998) A preliminary study of fauna and flora of a rice field in Kandy, Sri Lanka. Ceylon Journal of Science (Biological Science) 25:1-22.
- Bigirimana VP, Hua GKH, Nyamangyoku OI and Hofte, M (2015) Rice sheath rot: an emerging ubiquitous destructive disease complex. Frontiers in Plant Science 6:1-16. (Article No.1066)
- Chakraborty K, Moitra MN, Sanyal AK and Rath PC (2016) Important natural enemies of paddy insect pests in the Upper Gangetic plains of West Bengal, India. International Journal of Plant, Animal and Environmental Sciences 6(1):35-40.
- Coviella CE and Trumble JT 1999 Effects of Elevated Atmospheric Carbon Dioxide on InsectPlant Interactions. Conservation Biology 13(4): 700-712.
- Fakir GA (2000) An Annotated List of Seed-Borne Disease in Bangladesh. Seed Pathology Centre, Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensingh.
- Kaundal R, Kapoor AS and Raghava GPS (2006) Machine learning techniques in disease forecasting: A case study on rice blast prediction. BMC Bioinformatics 7:485.
- Krishnaiah K and Varma NRG (2018) Changing insect pest scenario in the rice ecosystem A National perspective accessed on 25.01.2018. p. 28.
- Manibhushanrao K and Krishnan P (1991) Epidemiology ofblast (EPIBLA): A simulation model and forecasting system for tropical rice in India. In: Rice Blast Modeling and Forecasting (Teng PS ed.). International Rice Research Institute, Philippines. pp. 31–38.
- Manikandan N, Kennedy JS and Geethalakshmi V (2013) Effect of elevated temperature on development time of rice yellow stem borer. Indian Journal of Science and Technology 6 (12):5563–5566.
- Matic S, Bagnaresi P, Biselli C, Orru L, Amaral Carneiro G, Siciliano I, Vale G, Gullino ML and Spadaro D (2016) Comparative transcriptome profiling of resistant and susceptible rice genotypes in response to the seed borne pathogen *Fusariu fujikuroi*. BMC Genomics 17:19.
- Mohapatra SD (2014) Rice IPM Biodiversity implication In: Compilation of Invited Lectures delivered in National Symposium on Entomology as a Science and IPM as a Technology – the way forward November 14-15, 2014 at College of Horticulture & Forestry, CAU, Pasighat, Arunachal Pradesh p. 333-346.



- Mohapatra SD, Rath PC and Jena M (2016) Sustainable management of insect pests and diseases in rice in rainfed low land ecosystem: A case study. Indian Journal of Plant Protection 44 (2):189-191.
- Nayak D, Bose LK, Singh UD, Singh S and Nayak P (2008) Measurement of genetic diversity of virulence in populations of *Xanthomonas oryzae* pv. *oryzae* in India. Communications in Biometry and Crop Science 3(1):16–28.
- Nessa B, Salam MU, Haque AHM, Mahfuzul, Biswas JK, Shahjahan MK, MacLeod WJ, D'Antuono M, Barman HN, Abdul Latif M and Galloway J (2015) Spatial pattern of natural spread of rice false smut (*Ustilaginoidea virens*) disease in fields. American Journal of Agricultural and Biological Sciences 10(2):63-73.
- NRRI Annual Report (2014-15). ICAR National Rice Research Institute, Cuttack.
- Pandi PGG, Chander S, Pal M, Pathak H (2016) Impact of elevated CO<sub>2</sub> and temperature on brown planthopper population in rice ecosystem. Proceeding of National Academy of Science. India Section. B. Biological Science. DOI 10.1007/s40011-016-0727-x.
- Parasappa HH, Narasa Reddy G and Neelakanth (2017) Rice insect pests and their natural enemies complex in different rice ecosystem of Cauvery command areas of Karnataka. Journal of Entomology and Zoology Studies 5(5):335-338.
- Savary S, Nelson A, Willocquet L, Pangga I and Aunario J (2012) Modelling and mapping potential epidemics of rice diseases globally. Crop Protection 34:6–17.
- Sun X, Kang S, Zhang Y, Tan X and Yu Y (2013) Genetic Diversity and Population Structure of Rice Pathogen Ustilaginoidea virens in China. PLOS One 8(9):e76879.
- Yugander A, Sundaram RM, Ladhalakshmi D, Hajira SK, Prakasam V, Prasad MS, Sheshu Madhav M, Ravindra Babu V and Laha GS (2017) Virulence profiling of *Xanthomonas oryzae pv. oryzae* isolates, causing bacterial blight of rice in India. European Journal of Plant Pathology DOI 10.1007/s10658-017-1176-y.\*



## Bio-intensive Management of Pest and Diseases of Rice

## AK Mukherjee, MK Bag, M Annamalai, T Adak, S Lenka, Basana Gowda G, Prasanthi G, Raghu S, MS Baite, Prabhukarthikeyan SR, NB Patil, PC Rath, GP Pandi G, RR Korada, N Basak, U Kumar, SD Mohapatra, S Bhagat, A Banerjee, R Bhagawati and M Jena

## SUMMARY

Biointensive approaches incorporating ecological and economic factors into agricultural system and addresses public concerns about environmental quality and food safety may be a sustainable approach. Different bio pesticides like biocontrol agents and botanicals may help in the reduction of crop loss due to pests and diseases. Different naturally occurring microbial agents which don't have adverse effect on crops but are toxic or growth retardant against the pathogen or insects have been used as biocontrol agents (BCAs). Similarly different secondary metabolites or crude plant extracts or oils which are not phytotoxic but toxic or repellant to the insects are also being used for protection of crops against insect pests. The major problem of using BCAs or botanicals for management of insect pests and diseases are that its viability, wide spread efficacy, specificity, mass multiplication and sufficient source of the product especially for the botanicals. However, extensive research works have been undertaken to overcome the aforesaid problems that are discussed in details.

## **1. INTRODUCTION**

Pests and diseases pose a serious threat to the rice production. To mitigate these problems a significant amount of pesticide is used in conventional rice production. This led to resurgence of newer biotypes/strains/isolates, development of acquired pesticide resistance besides environmental pollution and health hazards. The conceivable approach is therefore, the biointensive integrated pest management to minimize the problem of pest and diseases *vis-à-vis* increasing rice production and reducing environmental hazards.

'Biointensive' approaches actually incorporate ecological and economic factors into agricultural system and addresses public concerns about environmental quality and food safety. The benefits of implementing biointensive approaches include reduced chemical input costs, reduced on-farm and off-farm environmental impacts, and more effective and sustainable pest management. Biological control agents (BCAs) are one of the most important components of biointensive approaches for rice pest and disease management. Biological control of diseases and pests employs natural



enemies of pests or pathogens to eradicate or control such population causing economic loss. This can involve the introduction of exotic species, or it can be a matter of harnessing whatever form of BCAs exists naturally in the ecosystem in question. If a pathogen is kept in check by the microbial community around it, then biological control is achieved. Biological control appears to take place on the plant surface by the activity of epiphytic microflora. This is then an important consideration when applying chemicals to plants, since there is a risk of killing natural antagonists of pathogens other than the one being treated.

The BCAs include all kinds of biopesticides comprising living organisms and/or their products that are used to suppress and manage pest populations. Food and Agriculture Organization (FAO) has comprehensively put forth biopesticides as "A compound that kills organisms by virtue of specific biological effects rather than as a broader chemical poison". Whereas, CABI has defined in more lucid term as "Biopesticides use naturally occurring organisms, such as fungi, bacteria, viruses and nematodes to control plant diseases and arthropod pests". Environment Protection Agency (EPA) has defined biopesticides as certain types of pesticides derived from natural materials such as animals, plants, bacteria, and certain minerals. Biopesticides fall into two major classes: firstly active biomolecules from plant, animal and microorganisms and secondly microbial pesticides consist of a microorganism e.g. a bacterium, fungus, virus or protozoan as the active ingredient. Biopesticides may be bio-fungicides, bio-insecticides, bio-nematicides, entomophillic nematodes and biorationals etc. Besides its controlling ability of pests and diseases, it has several advantages like lower exposure, quick decomposition, remains virtually no residues and allowing field for next crop immediately after application in the previous crop. Biopesticides has expanding areas in the global pesticide market and is likely to grow at a 15.6% compound annual growth rate (CAGR) from \$1.6 billion in 2009 to \$3.3 billion in 2014 (Ken Research Report 2015). About 63 Indian private companies altogether registered 970 products. Indian government also intervened in this matter and ICAR has 31 BCA production facilities while Department of Biotechnology supported 22 and the Insecticide Act of 1968 was also simplified registration procedure for speedier development of biopesticides. Demand for nature-based biopesticides is increasing steadily in all over world because of increased environmental awareness and the pollution potential and health hazards from many conventional pesticides. Thus popularity of Biological control agents has increased significantly in recent years, as extensive and systematic research has enhanced their efficacy. Several research centres around the world are conducting research aimed at improving techniques for the augmentation and application of biological control agents, with the objective of getting better commercial and ready to use products. The National Farmer Policy, 2007 is also treated at par with chemical pesticide for promotion and utilization of BCAs. In the present book chapters authors have reviewed the use of different BCAs, their efficacy, their adaptability, development and use of suitable formulations of BCAs that provide higher competitive saprophytic ability to the BCAs which help in maintaining viability of the BCA for longer duration.



## 1.1. Modus operandi of biological control agents

The most common mechanisms for microbial antagonism of plant pathogens are parasitism, predation, competition, induced resistance and production of antimicrobial substances. Often, several mechanisms act together.

Competition exists between organisms that require the same resource for growth and survival. Competition for space or nutrients usually takes place between closely related species. Therefore, it can be effective to treat plants or seeds with a nonpathogenic strain of a related species that can out-compete the pathogenic organism or the treating species need not be closely related to the pathogen, as long as it uses the same resources.

Parasitism be it hyperparasitism or mycoparasitism is well documented and is affected by environmental factors, including nutrient availability. Formulations of some parasitic species of fungi are available commercially for the control of fungal plant pathogens in the soil and on the plant surface. Bacteria on the plant surface and in the soil are also known to parasitize plant pathogens. Predation of plant pathogens by invertebrates can also contribute to general biological control. Bacterial feeding nematodes consume large numbers of bacteria in the soil and some amoebae are known to attack yeasts, small spores and fungal hypha, although these organisms are generally non-specific predators and their relative importance in biological control is not well understood.

Induced resistance and cross-protection are two mechanisms of plant 'immunity' against a pathogen. In the case of cross-protection, an organism present on the plant can protect it from a pathogen that comes into contact with the plant later. Induced resistance is a form of cross-protection, where the plant is inoculated with inactive pathogens, low doses of pathogens, pathogen-derived chemicals or with non-pathogen species to stimulate an immune response. This prepares the plant for an attack by pathogens, and its defense mechanisms are already activated when infection occurs. It provides protection against a wide range of pathogens across many plant species.

## 2. RESEARCH STATUS OF BIOLOGICAL CONTROL AGENT

## 2.1. Rice diseases

Sheath blight (ShB) of rice caused by *Rhizoctonia solani* Kuhn {*Thanatephorus cucumeris* (Frank) Donk} is one of the serious diseases and is prevalent in almost all high yielding rice varieties growing area in India. A modest estimation of losses due to sheath blight in India has been reported up to 54.3%. Currently, the disease is managed mostly by application of systemic fungicides. No genetic resistance has been reported for this disease and all the rice cultivars are susceptible to the pathogen. As an alternative, biological control of plant pathogens gaining popularity in majority of crops, its utilization in rice ecosystem is still at its infancy due to varied reasons.



Rice, being a crop that is grown under inundated conditions, the survival, growth and establishment of BCAs is questionable. However, effective management strategy of sheath blight disease is feasible only when the BCAs those are in vogue in rice based cropping systems survive, establish, proliferate and control sheath blight pathogen and also have a synergistic growth promoting effect on the crop. Besides, the BCAs should be able to induce systemic resistance thereby contributing to the disease control. Bio-control agents/antagonists are considered as one of the effective and eco-friendly means of management of diseases in different crops. Several fungi like *Trichoderma viride, T. harzianum, T. koningii* (Das and Hazarika 2000); *Aspergillus niger, A. terreus, Gliocladium virens* of rice field are found to be antagonistic against *R. solani* (Gogoi and Roy 1993).

Among the fungal antagonists, *Trichoderma* spp. and *Gliocladium* spp. are widely used in the management of rice ShB disease. These fungal antagonists are either applied to rice seed, soil, as root dip and foliar spray for managing the disease. Foliar application of *Trichoderma* spp. also was found very effective in reducing ShB severity. Studies on field application of *T. harzianum*as talc + CMC based formulation reduced disease severity by 52%. The bioagent was found effective when applied at 7 days compared to simultaneous application with ShB pathogen (Khan and Sinha 2006). The optimum dose of the bioagent was found to be 4 or 8 g L<sup>-1</sup> and increased grain yields were also reported (Khan and Sinha 2007). Spray application of the bioagent was highly effective on rice seedlings that received 60 kg N + 60 kg P + 40 kg K /ha. Further, Trichoderma spp. isolated from rice leaf was more effective compared to T. virens isolated from rhizosphere (Khan and Sinha 2005). Mixed mode of application of bioagent as soil treatment, root dipping, and foliar spray was found to be very effective in reducing ShB severity over control. However, foliar application of the bioagent alone was also effective under field conditions. Nagaraju et al. (2002) reported that application of T. viride as root dip + spray was effective in reducing ShB severity by 59% under field conditions

Mathivanan et al. (2005) reported that combined applications of *T. viride* and *Pseudomonas fluorescens* was effective without any negative effects in reducing rice ShB besides increasing number of productive tillers, higher grain and straw yields. However, individual applications of bacterial and fungal antagonists separately had more beneficial effects. Bhagawati and Roy (2005) proved that ShB disease suppression at field level can be obtained by soil application of *T. harzianum* and *T. viride* at a pH range of 5.1 to 6.0. A concomitant increase in plant growth and yield was obtained. Further, it was reported that population levels of *Trichoderma* spp. are high and that of *R. solani* are low in acid soils.

Among the bacterial biocontrol agents, plant growth-promoting rhizobacteria (PGPR) offer a promising means of controlling plant diseases besides contributing to the plant resistance, growth and yield in rice (Mew and Rosales 1992). Of the different PGPR, fluorescent *Pseudomonas and Bacillus spp.* group of bacteria offer an effective control of ShB besides inducing growth promoting effects and systemic resistance. Bacteria isolated from rice seeds and rice ecosystem was able to effectively suppress



ShB besides producing growth promoting effects. Two years pooled data of a field experiment revealed that *P. fluorescens* product gave significant result over untreated control but at par with carbendazim 50WP (check fungicide) treated plot (Bag and Bandyopadhyay 2010). Seed treatment with these antagonistic bacteria resulted in increased root and shoot length of seedlings.

*Bacillus* spp. are important gram positive PGPR in the biocontrol of rice ShB disease. The bacterium produces endospores and microscopic studies revealed that isolates of *B. subtilis* and *B.megaterium* exhibited effective inhibition against the pathogens of ShB and bakane diseases of rice. The fermented product of *Bacillus* strain Drt-11 was highly antagonistic to rice ShB pathogen, causing reduced sclerotial germination (40-60% inhibition over control), reduced hyphal growth and colony diameter (by 14%) besides increased rice seedling growth (Min and Hui 2006).

Sheath Rot (ShR) of rice is another emerging disease of rice caused by Sarocladium oryzae now observed in almost all rice-growing ecosystems of the world and causing yield losses of 3-85% depending on disease severity and complete suppression of panicle exertion (Sundaramoorthy et al. 2013). In recent years, Fusarium species and bacterial pathogen Pseudomonas fuscovaginae have been found to be associated with rice sheath rot in making it more complex disease. The different species of Fusarium forming complex are F. fujikuroi, F. verticillioides and F. proliferatum cause various symptoms on different plant parts and are responsible of yield losses of 40% in Nepal (Desjardins et al. 2000) and even upto 60% in Korea (Park et al. 2005). Soil has enormous untapped potential antagonistic microbes i.e. Bacillus, fluorescent pseudomonads and Trichoderma spp. Among them, Bacillus species have received the most attention due to their antimicrobial and surfactant properties (Gross and Loper 2009). Bacillus producing cyclic lipopeptides (CLPs) of the surfactin, iturin and fengycin families and their antimicrobial activities are well studied (Vinodkumar et al. 2017).

The combination of PGPR strains was more effective in reducing sheath rot disease in rice plants compared to individual strains under glasshouse and field conditions (Saravanakumar et al. 2008; Sundaramoorthy et al. 2013). Bag et al. (2010) observed that a *P. fluorescens* product commercially available in the market reduced ShR disease incidence significantly over the untreated control and at par with the check fungicide. While another *Trichoderma* based BCA and *Gaultheria* extract based botanicals also reduced the disease incidence, but below the fungicide check. The investigations on induced systemic resistance (ISR) by PGPR demonstrated that several strains protect plants from the plant diseases through the activation of defense genes and expression of stress-related proteins. These induced defense responses are regulated by a network of interconnecting signal transduction pathways viz., salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) which play key roles in activating defense genes encoding peroxidase, polyphenol oxidase, catalase, superoxide dismutase, chitinase, â-1,3-glucanase, lipoxygenase, proteinase inhibitors and phenylalanine ammonia lyase (Van Loon et al. 2008).



Bakanae or foolish seedling disease of rice caused by soil borne fungus Fusarium *fujikuroi*, another emerging disease of rice causes yield losses of 20-50% in Japan, 15% in Eastern India and 3.7-14.7% in Thailand. Biocontrol agents like Trichoderma releases a variety of compounds peroxidases, chitinases, b-1, 3-glucanases, lipoxygenase-pathway hydro peroxide lyase and compounds like phytoalexins and phenols that induce resistance responses to biotic and abiotic stresses (Harman et al. 2004; Cardona and Rodriguez 2006). Root colonization by Trichoderma harzianum results in increased level of plant enzymes. Trichoderma has proved its efficacy to control Fusarium fujikuroi (Hadwan and Khara 1990; Lin et al. 1994). P. fluorescens and B. cereus isolates effectively control rice bakanae and foot rot disease when it was applied to the seed, soil or as foliar spray (Kazempour and Elahinia 2007; Zhang et al. 2010). Hossain et al. (2016) have shown that, root drenching with an endophytic strain of Bacillus oryzicola YC7007 suspension reduced the disease severity of bakanae significantly when compared with the untreated controls. Lakshmi Kumari et al. (1972) observed that thermolabile, ether soluble fungistatic substance produced by Azotobacter spp. inhibited growth and conidial germination of F. moniliforme. Seed treatment and soil incorporation of Pseudomonas aureofaciens and other antagonistic bacteria suppressed the growth of the pathogen (Lee et al. 1990). Kumar et al. (2007) reported that P. fluorescens isolates PF-9, PF-13 and B. thuringiensis isolate B-44 significantly reduced the fungal growth and bakanae incidence. Seed treatment has been found more effective than spraying antagonistic isolates and their efficacy was improved by combining the biological control agents (Lu et al. 1998). But still there is a lot of scope to identify new and potential biocontrol agents and elucidating their role in interaction and control of bakanae disease.

False smut caused by *Ustilaginoidea virens* (Che.) Tak. (teleomorph *Villosi clava virens*) is a serious disease of rice worldwide. Yield loss due to false smut ranged from 0.2 to 49.0 per cent in India (Dodan and Singh 1996). Presently, the control of rice false smut disease mostly relies on fungicides. However, heavy reliance on fungicides is not only harmful to the environment, but also increases the expenses for crop production. Therefore, exploration of BCAs for management of false smut disease is highly needed. Reports available from scanty literatures revealed that *Trichoderma viride* have antagonistic potential against *U. virens* (Kannahi et al. 2016). Bioagent, *B. subtilis* was reported to be effective against the disease (Liu et al. 2007).

## 2.2. Status of botanicals against rice insect pests

A study conducted by ICAR- NRRI in tribal areas of eastern India indicated that, the insect pests of rice like yellow stem borer, brown plant hopper, case worm, gundhi bug and other pests were effectively managed through the use of botanicals as indigenous technology. They are mainly based on direct application of different plant parts of neem (*Azadirachta indica*), karanja (*Pongamia pinnata*), parasi (*Cleistanthus collinus*), mahua (*Madhuca indica*), kochila (*Strichnos nux vomica*), harida (*Terminalia chebula*), sal (*Shorea robusta*), begunia (*Vitex nigundo*), wild sugarcane (*Sachharum spontaneum*), turmeric and also organic matters like cow dung (Jena



2012). Apart from direct application, the ITK-based botanical like Panimirch, *Polygonum hydropiper* used as fish toxicant by the tribals, was also found effective in controlling the notorious rice pests like BPH and case worm. Its toxicity was assessed against fingerlings of *Catla catla* along with neem oil under rice-fish farming system research (Das et al. 2005). The ITKs on neem, karanja, kochila, parasi and wild sugarcane were evaluated in detail and their efficacy was ascertained (Jena and Dani 1994; Jena 1997; Jena and Dani 1997; Jena 2000; Jena 2005; Jena and Behera 2008). The validation of ITKs on Parasi against YSB, case worm, gundhi bug and wild sugarcane against case worm, were tested in the farmers' field (Jena and Behera 2004; Jena and Dangar 2004). Synergistic action of neem oil for enhancing the efficacy of insecticides was also studied (Jena et al. 2004).

Methanol extract of Thevetia nerifolia leaf were evaluated against Spodoptera *litura* (Fab.) and the results showed 53.8% larval mortality and only 29.6% pupation and 22.3% adult emergence at 2.5% concentration level. Sub-fractioned extract with solvents of different polarity indicated chloroform extract active in terms of increased larval mortality (27.5-61.5%), reduced pupation (28.4-60.2%) and adult emergence (19.8-52.8%) and the activity was found to be attributed to the glycosides present in the extract (Ray et al. 2012). Leaf, stem and root extract of *Thevetia peruviana* (Pers) Schum. in four organic solvents; petroleum spirit, ethyl acetate, acetone and methanol on the adults of Callosobruchus maculatus F. effectively produced mortality and their toxicity was in the order of solvents: petroleum spirit>ethyl acetate>acetone>methanol and among the extracts, root extract was most toxic to C. maculates (Mollah and Islam 2007). The antimicrobial potential of 50% ethanolic extract of T. peruviana (kaner) leaves against some micro-organisms like Staphylococcus aureus, Rhizobium sp., E. coli and Streptococcus sp. indicated that the phytochemical extracts of T. peruviana exhibited significant activity at varying dosages (50-150 mg/ml). It revealed that 50% ethanolic extract of T. peruviana leaves can be used as a potential source of novel antibacterial agents against E.coli and S. aureus (Naza and Agrawal 2015). Thus testing of *Thevetia* against leaf folder of rice will give a new look on control of this insect pest.

Yellow stem borer, *Scirpophaga incertulas* is one of the most important insect pests attacking rice from seedling to harvest stage. Farmers depend upon a large number of insecticide applications, even though a lot of insecticide applications are not effective. In recent years the use of synthetic insecticides in crop protection programme around the world has resulted in disturbance in eco-bio-balance, pest resurgence, pest resistance to pesticides and lethal effect to non-target organisms in the agro-ecosystems in addition to direct toxicity to users. Therefore, it has now become necessary to search for the alternative means of pest control, which can minimize the use of synthetic insecticides. Botanical pesticides and several botanicals have been reported as pesticides, antifeedants, insect growth regulators and repellents (Jena 2012; Mishra 2014). Many botanicals were found effective against various rice pests is listed in Table 1.



#### Table 1. Botanicals tested against major rice pests.

S. No.	Botanicals and their efficiency	Dice pest	Authors
	Botanicals and their efficacy	Rice pest	Authors
1	Neem + Mahogony oil performed best in reducing dead heart and white ear damage	Stem borer	Majlish et al. (2014)
2	In a field experiment, botanical neem extract reduced the dead heart to the tune of 58.08% compared to control.	Stem borer	Islam et al. (2013)
3	Neem seed kernel showed significant reduction of stem borer damage (number of white ear heads)	Stem borer	Ogah et al. (2011)
4	Pongamia pinnata, Nicotiana tabaccum, Citrus grandis	Stem borer	Barman et al. (2014)
5	Seed extract of Annona squmosa, Sapindus trifoliates, Acacia concinna, Hydrocarpus alpine, Gynandropsis pentaphylla and Ocimum Gratissimum bring down the oviposition rate	ВРН	Reddy and Urs (1988)
6	Oil and seed extracts of <i>Pongamia</i> glabra and Madhuca longifolia reduce hatching rate	BPH and WBPH	Ramanuj and Sundarababu (1989)
7	Leaf extract of <i>Anona reticulate</i> and vine extracts of <i>Tinosphora rumphii</i> showed repellant action	ВРН	Telan et al. (1994)
8	Leaf extract of <i>Andrographis paniculata</i> and <i>Coleus aromaticus</i> protect the grain	Gundhi bug	CRRI Annual report 1995-96
9	Seed oil extracts of <i>Annona squamosa</i> and mahua ( <i>M. longifolia</i> ) caused mortality	GLH	Narsimham and Mariappan (1988)
10	Plant essential oils of <i>Eucalyptus</i> sp found to show contact, fumigant and persistent toxicity and repellency activity	Rice weevil	Patil et al. (2016)

The major limitation in botanical pesticides' usage is that their active principles are easily degraded by the sun light or UV radiations and heat in open field conditions (Ware and Whitacre 2004). Recent studies have shown that polymeric systems can be used for the protection of pesticide molecules from natural degradation and for its controlled release against the target pests (Campos et al. 2014). To avoid higher initial dosages or repeated applications, many attempts were made to control the release rate of biocides by encapsulating them mainly in polymeric carriers. Both biodegradable and synthetic polymers can be applied as release rate controlling barrier materials of biocides; however, the polymeric carriers are expensive due to their synthesis and precursors, and they are thermally, dimensionally and chemically unstable.

For such protective and slow release, the following materials can be used.

 Biogenic silica nano particles could be used as an attempt to design cheaper and cleaner controlled release systems compared to those prepared with polymeric and synthetic silica as carrier material.



• The nano particles of chitosan have good biocompatibility with other molecules and have the potential for controlled release of the active molecules.

## 2.3. Infochemicals or semiochemicals for management of rice insect pests

In recent years, to combat pest damage, the pest management tactics using behavioural manipulation, including mating disruption, feeding disruption, oviposition deterrence, use of attractants, and pre-release training, have become the focus of research for pest control (Roitberg 2007; Phillips 1997). Successful mating disruption technique has been demonstrated for pyralid storage moths under simulated storage experiments (Sower and Whitmer 1977). Mating disruption, which prevents males from finding females, is the most widely studied area of behavioural manipulation for pest management (Roitberg 2007). Female moths emit a volatile pheromone that is detected by males at distance to locate the sexually receptive female, and male antennae have a large number of sensilla that contain olfactory receptor neurons specific to components of the female sex pheromones (Schlamp et al. 2006). Ultrastructure of antennal and ovipositor sensilla of S. cerealella and the location of the female sex pheromone gland was determined by Ma et al. (2017). Seven types of antennal sensilla were identified on both sexes, out of them the Sensilla trichodea was found significantly more abundant on male antennae than on those of females, suggesting that these sensilla may detect the sex pheromones. On the ovipositor, only Sensill achaetica of various lengths was found. The sexual gland was an eversible sac of glandular epithelium, situated dorsally in the inter-segmental membrane between the 8th and 9th abdominal segments (Ma et al. 2017). The pheromone X-lure was found useful only for monitoring S. cerealella (Akter and Ali 2016). Host-finding efficiency of natural enemies in biological control programs could be improved with the use of kairomones in mass-rearing or release protocols.

Among the stored grain pests, Angoumois grain moth, *Sitotroga cerealella* is considered to be most destructive pests of cereal grains worldwide, particularly in the tropics and warm temperate regions (Trematerra 2015). Its infestation starts in the standing crop and continues in storage. Although there are many control strategies, some effective, cheap and readily available strategy are the present need for safe storage. Several approaches for managing *S. cerealella* include use of edible oils, containers, synthetic chemicals, agricultural waste materials, plant derivatives, bacterial protoxins, biopesticides, biocontrol enhancers and semiochemicals. Among these methods, till now semiochemicals have not been identified for *S. cerealella*. By feeding inside grains, *S. cerealella* is directly protected from chemical insecticides and causes damage in both the field and storage condition (Fouad et al. 2014).

Gundhi bugs are also called stink bugs. An understanding of preference of gundhi bugs to the host and mating behavior of adults is essential to identify semiochemicals (infochemicals) to devise a trapping system. A detailed review on rice stinkbugs was done by Litsinger et al. (2015). To detect a host plant, gundhi bugs first have to land on it to determine whether the plant is of a preferred species or quality. Gunawardena

and Ranatunga (1989) found gundhi bugs are attracted to the host plants probably by odor, based on tests of steam distillates of milk-stage panicles. Kainoh et al. (1980) using flight-tunnel experiments, suggested that adults can detect the odor of rice plants when they fly close to a rice field, and aggregation on the panicles was mainly due to an arrestant effect, exerted by the panicles at flowering. Adults examine the plant closely through plant surface exploration using sensory apparatus such as antennae where they walk about and/or tapping with the labium tip onto the surface of the plant (Ishizaki et al. 2011).

Leal et al. (1996) pointed out that few out of the 35,000 species of Heteroptera worldwide had sexual pheromones. The copious defensive secretions that contaminated samples have hampered progress on gundhi bugs. A defensive alarm pheromone has been identified from a related species, the bean bug *Riptortus clavatus*, as well as an aggregation pheromone that enables second instar nymphs to find the host food plant. In addition, bean bug males release semio-chemicals that attract both males and females. Interestingly, the case of the gundhi bugs is just the opposite i.e. only males are attracted to semio-chemicals that are produced and emitted by both males and females. Although *L. chinensis* females can detect the male attractants, do not elicit any attraction. Thus, both sexes produce attractants, but these are not true sex pheromones.

Farmers also have used various materials that act as repellents to ward off gundhi bugs. Farmers in Assam, India spread goat dung in rice fields (Deka et al. 2006), while tribals of Arunachal Pradesh placed dried pomelo leaves in the field (Saravanan 2010). Lefroy (1908) heard that farmers in Sri Lanka burn certain aromatic herbs and resinous substances so the wind will carry the smoke into the rice crop to repel gundhi bugs 'with considerable success'. A survey found 15% of farmers in Claveria, Philippines burnt grass or rubber tyres and 2% burnt animal fat (goat) as repellents (Litsinger et al. 2009). Other farmers set bonfires to repel the bugs by burning obnoxious plants like *Annona squamosa, Derris elliptica*, betel nut *Areca catechu, Gliricidia sepium, Erythrina variegata, Pittosporumres iniferum, Pongamia pinnata,* and *Wikstroemia ovata*.

Many species of Alydidae that are attracted to pig carcass (Schaefer et al. 1983) aggregate on rotting meat (Uichanco 1921) and the farmers used this practice to attempt to control gundhi bugs. Gundhi bug adults were attracted to rotten fish, mollusks, or shrimp (Otanes and Sison1941), dog meat or starfish (Litsinger et al. 2009), frog meat (Morrill et al.1991). Guimba et al. (2006) stated farmers to use golden apple snail *Pomacea canaliculata*. Guimba et al. (2006) used baffle traps with rotting meat and collected most of the gundhi bugs during flowering, but the numbers were low.

## 2.4. Grain volatiles for attraction of Angoumois grain moth, Sitotroga cerealella

To protect grains from the insect damages, tons of insecticides have been applied to control the pests, which have resulted in environmental damage, pest resurgence,



pest resistance to insecticides and lethal effects on non-target organisms. Furthermore, because of cost, these pesticides are becoming increasingly inaccessible to the farmers, particularly in developing countries. This fact, combined with the consumer's demand for residue-free food, prompted researchers to evaluate other alternative reduced-risk control methods for stored-grain protection (Tang et al. 2009). To resolve the above problems, new environmental friendly methods to control the insect damages have become of utmost interest to the researchers.

Phytophagous insects generally utilize volatile semiochemical cues from the host plants during one or more phases of the host selection process. Plant semiochemicals may act as direct attractants for insects or they may synergistically enhance the activity of pheromones produced by insects. However, little is known about interactions among rice grain and insect herbivores. Stored-product insects present a special situation for research on host plant volatiles because nearly all species are intimately adapted to human-stored grains and grain products, truly nonanthropogenic populations may not exist (Lindsley 1944).

Angoumois grain moths are serious destructive stored insect pests and frequently found flying haplessly in stored house. Larvae penetrate seed coat and enter the grain. In order to manage this pest through non-chemical especially semiochemical approach, Vick et al. (1974) have identified sex pheromone components of this moth. Subsequently several attempts have been made to improve the catching performance of adult moths, but somehow results were not found satisfactory (Akter and Ali 2015). Volatile attractants for several beetle species that infest broken grain have been identified from cereal grains and their products (e.g., Mikolajczak et al. 1984; Nara et al. 1981; Pierce et al. 1990). Work on the maize weevil, *Sitophilus zeamais*, demonstrated that odors from cracked wheat synergistically enhanced responses to male-produced pheromone (Walgenbach et al. 1987). Based on the previous works, we are interested to examine the behavioral activity of several grain-derived volatiles as attractants for improving attraction and catching efficiency of moth.

## 2.5. Novel formulation of botanicals and bioagents for better efficacy and higher shelf life

Of the estimated 0.5 million plant species that exist globally, nearly 10% have been examined chemically and over 6,500 screened for anti-pest properties. In India, products based on only three plants are registered under the Insecticides Act, 1968 (Table 2) (Parmar 2010).

Pesticide	Key source plant
Pyrethrum	Chrysanthemum sp. (ex. Cinerariaefolium, Coccinium etc.)
Neem	Azadirachta indica
Nicotine (Export only)	Nicotiana sp.

## Table 2. Botanical pesticides registered in India under the Insecticides Act, 1968.



Microbials as biopesticides include bacteria, fungi, nematodes, protozoa, viruses etc. and the mass released macrobial parasitoids and predators which are used for pest management. Their application is by inundative or inoculative means. The microbials in use include bacteria (ex. *Bacillus thuringiensis* (Berliner), entomopathogenic viruses (ex. nuclear polyhedrosis virus (NPV) and granulosis virus (GV), entomopathogenic fungi, eg. *Beauvaria bassiana*, and others. A total of 13 products based on bacteria, fungi and virus are registered for use in India (Table 3).

Organism	Formulation
Bacteria	
Bacillus sphaericus	1.15%WP
Bacillus thuringiensis var. israelsensis	5% WP, 5% AS
Bacillus thuringiensis var. kurstaki	5 & 7.5% WP
Pseudomonas fluorescens	0.5& 1% WP
Fungi	
Ampelomyces quisqualis	2% WP
Beauveria basssiana	1, 1.15, or 2.15% WP, 10% SC
Metarhizium anisopliae	1 & 1.5% WP
Trichoderma harzianum	0.5, 1 & 2% WP
Trichoderma viride	1% WP
Verticillium lecanii	1.15% WP
Virus	
Hear NPV	0.43, 0.5, 0.64 & 2% AS
Spli NPV	0.5 & 2% AS

#### Table 3. Microbial biopesticides registered for use under Insecticides Act, 1968.

WP= Wettable Powder, AS= Aqueous Solution, SC= Suspension Concentrate, WS= Slurry for Seed Treatment

(Wahab and Manjunath 2009)

## 3. KNOWLEDGE GAPS

The BCA formulations used India are based on mainly few microorganisms with comparatively specific antifungal/antibacterial activity so there is a need for identification of BCA with broad spectrum antifungal/antibacterial activity.

Application and grower acceptance of BCAs have been found slow to develop, mainly due to the variation in efficacy under the range of environmental conditions, likely to occur in the field. Better understanding of environmental parameters that limit biological control is required.

Most of the formulations available in India are wettable powder and are clay based. So, it reduces longevity of the active microbes by desiccating and by acting as abrasive agent. These formulations also do not protect the active molecules from external heat and UV light. These are having a very limited shelf life.

It has been indicated that slow progress in research on formulation and delivery systems is a major hurdle to the development of biopesticide products. Besides the



registration process for BCA in India is too complicated and at par with that of chemical pesticide which needs to be changed.

# 4. RESEARCH AND DEVELOPMENT NEEDS AND THE WAY FORWARD

It is essential to identify BCA which should have broad-spectrum antifungal/ antimicrobial activity and also should be effective over different geographical regions. It is important to develop biocontrol formulation in such way that the BCA remains viable, active and infectious for longer period but yet in dormant stage, safe, and easy for application. The dormancy of bioactive molecules is exogenous, not constitutive, so the key to prolonging their survival is to stop germination and to reduce metabolism as much as possible. One possibility for stabilization is dehydration. Another problem with biopesticides is limited shelf life due to its degradation in presence of UV light, moisture, pH, etc. Economic feasibility of these product depends on market size and spectrum of pests affected by the BCA, variability of field performance, costs of production, and a number of technological challenges, including fermentation, formulation, and delivery systems.

Essential oils have active ingredients towards many different biological activities like antimicrobial against pathogens, repellents and antifeedent properties against insect pests. These naturals can be exploited effectively using nano-emulsion technology or the active ingredients can be released in a controlled fashion from the nano-biomaterials. Similarly formulation can be taken up to have floating bioagents to survive rice ecosystem. Controlled release technology will give a proper coat on the biopesticides to render limited expose to environment. Limited exposure and sustained release of biopesticides from controlled release matrices will be most preferable.

Currently there is a lack of studies concern to the loading of active compounds in inorganic supports such as the silica nanostructures and also, biogenic silica nanostructures have not been explored as release rate controlling material of biocides. Considering these both scientific gaps, the study concerning using biogenic silica nanostructures as support for active compounds can provide an alternative for green, controlled-release biocides.

#### References

- Akter T and Ali M (2015). Efficacy of pheromone on capturing angoumois grain moth (*Sitotroga cerealella*) in stored rice (*Oryza Sativa* L.) grain. The Agriculturists 13(2):74-80.
- Bag MK and Bandyopadhyay P (2012). Evaluation of bioefficacy of botanicals and biopesticides against sheath blight disease of rice. Journal of Mycopathological Research 50(1): 101-103.
- Bag MK, Roychoudhury UK and Adhikari B (2010). Efficacy of botanicals and biopesticedes against sheath rot disease of rice in West Bengal. Journal of Crop and Weed 6(2): 82-83.



- Bhagawati R and Roy AK (2005). Effect of different moisture regimes on survival of *Trichoderma harzianum* and *Trichoderma viride* and development of sheath blight under pot condition. Oryza 42(2): 133-137.
- Boshra SA (2007). Effect of high-temperature pre-irradiation on reproduction and mating competitiveness of male *Sitotroga cerealella* (Olivier) and their F-1 progeny. Journal of Stored Products Research 43: 73–78.
- Cardona R and Rodriguez H (2006). Effect of *Trichoderma harzianum* fungus on the incidence of the charcoal rot disease on sesame. Revista de la Facultad de Agronomía (LUZ) 23: 42-47.
- Das BC and Hazarika DK (2000). Biological management of rice sheath blight. Indian Phytopathology 53(4): 433-435.
- Fouad HA, Faroni LRD, Tavares WS, Ribeiro RC, Freitas SS, Zanuncio JC (2014). Botanical extracts of plants from the Brazilian Cerrado for the integrated management of *Sitotroga cerealella* (Lepidoptera: Gelechiidae) in stored grain. Journal of Stored Products Research 57: 6–11
- Harman GE (2006). Overview of mechanism and uses of *Trichoderma* spp. Phytopathology 96: 190-194.
- Harman GE, Petzoldt R, Comis A and Chen J (2004). Interactions between *Trichoderma harzianum* strain T22 and maize inbred line Mo17 and effects of this interaction on diseases caused by *Pythium ultmum* and *Colletotrichum graminicola*. Phytopathology 94(2): 147-153
- Ishizaki M, Watanabe T, Moriya S, Tabuchi K (2011). Diurnal locomotion activity of adultrice bug, *Leptocorisa chinensis* (Hemiptera: Alydidae), at different ages, measured by actinograph and video camera. Applied Entomology and Zoology 46: 135-142.
- Jena M (2005). Integrated pest management with botanical pesticides in rice with emphasis on neem products. Oryza 42(2):124-128.
- Jena M and Dani RC (1997). Evaluation of some neem based formulations as oviposition deterrent and ovicides against brown plant hopper and yellow stem borer of rice. Oryza 34(3): 280-282.
- Jena M (1997). Efficacy of some neem products against rice leaf folder, Cnaphalocrocis medinalis Guenee. Indian Journal of Plant Protection 25(2):160-161.
- Khan AA and Sinha AP (2007). Biocontrol potential of *Trichoderma* species against sheath blight of rice. Indian Phytopathology 60: 208-213.
- Kumar MN, Laha GS and Reddy CS (2007). Role of antagonistic bacteria in suppression of bakanae disease of rice caused by *Fusarium moniliforme* Sheld. Journal of Biological Control 21(1): 97-104.
- Leal WS, Ueda Y, Ono M (1996). Attractant pheromone for male rice bug, *Leptocorisa chinensis*: Semiochemicals produced by both male and female. Journal of Chemical Ecology 22: 1429-1437.
- Lee YH, Shim, GY, Lee EJ and Mew TW (1990). Evaluation of biocontrol activity of fluorescent pseudomonads against some rice fungal diseases *in vitro* and green house. Korean Journal of Plant Pathology 6(1): 73-80.
- Ma Min, Chang Meng-Meng, Lu Yan, Lei Chao-Liang and Yang, Feng-Lian (2017). Ultrastructure of sensilla of antennae and ovipositor of *Sitotroga cerealella* (Lepidoptera: Gelechiidae), and location of female sex pheromone gland. Scientific Reports 7: 40637. DOI: 10.1038/ srep40637.



- Park JW, Choi SY, Hwang HJ and Kim YB (2005). Fungal mycoflora and mycotoxins in Korean polished rice destined or humans. International Journal of Food Microbiology 103: 305– 314.
- Saravanakumar D, Lavanya N, Muthumeena K, Raguchander T and Samiyappan R (2008). Fluorescent pseudomonad mixtures mediate disease resistance in rice plants against sheath rot (*Sarocladium oryzae*) disease. Biological Control 54: 273-286.
- Sower LL and Whitmer GP (1977). Population growth and mating success of Indian meal moths and almond moths in the presence of synthetic sex pheromone. *Environmental Entomology* 6: 17–20.
- Sundaramoorthy S, Karthiba L, Raguchander T and Samiyappan R (2013). Eco-friendly approaches of potential microbial bioagents in management of sheath rot disease in Rice caused by *Sarocladium oryzae* (Sawada). The Plant Pathology Journal 12: 98-103.
- Tang QF, Wu YJ, Liu BM and Yu ZL (2009). Olfactory responses of *Lariophagus distinguendus* (Hymenoptera: Pteromalidae) to volatile signals derived from host habitats. The Philippine Agricultural Scientist 92: 133-142.
- Van Loon LC, Bakker PAHM, Walter HW, Van der Heijdt WHW, Wendehenne D and Pugin A (2008). Early responses of tobacco suspension cells to rhizobacterial elicitors of induced systemic resistance. Molecular Plant-Microbe Interactions 21: 1609-1621.
- Wahab S and Manjunath TM (2009). Biopesticides: Analysis of constraints and strategies for future growth. Department of Biotechnology, Government of India. 42 p.
- Zhang SM, Sha CQ, Zhao XY, Wang YX, Zhang XC, Li J, Meng LQ and Yu DS (2010). Identification of an endophytic *Paenibacillus polymyxa* strain producing antifungal protein and the inhibition to *Fusarium moniliforme* causing rice bakanae disease. Chinese Journal of Biotechnology 30: 84–88.<sup>⊕</sup>



## **Optimization of Chemical Pesticide use in Rice**

## PC Rath, T Adak, M Jena, MK Bag, Raghu S, Annamalai M, MS Baite, NB Patil, Prasanthi G, U Kumar, P Panneerselvam, GP Pandi G, S Lenka, Basana Gowda G, SD Mohapatra, AK Mukherjee, Aravindan S, MK Yadav, Prabhukarthikeyan SR and K Sankari Meena

## SUMMARY

Pesticide is an indispensible part of modern agriculture. Over the years, new researches from private and public organization are towards developing new molecules or new formulations which are easy to use, economic and environmentally safe. The pesticide poisoning and pollution are two major negative effects of pesticides. Awareness programme should be included to obtain the optimize pesticide use. Proper pest monitoring, protective clothing, application of pesticide at right time at right dose and at right quantity should be integral part of pesticide application. Integrated pesticide resistance management should be included in farm practices and both private and public organization should take active participation in managing the problem. Otherwise, there is a great chance that we may not have any pesticide option left in pest management in near future. Genuine concerns on consumer and environmental safety of pesticide uses should be dealt with scientific findings. Need of the hour is to have a readymade pesticide detection kit at affordable price. Long term pesticide uses and its effect on flora and fauna should be investigated and should be included in cost-benefit ratio calculation.

## **1. INTRODUCTION**

In India, around 40 per cent of the total cultivated area is treated with the pesticides. Approximately, 65-70 per cent of the cultivated area treated with pesticides is irrigated. The production of pesticides started in India in 1952 and at present, India is the fourth largest global producer of agrochemicals after the US, Japan and China. These pesticide industries had a value of USD 4.4 billion in financial year 2015 and are expected to grow at 7.5% per annum to reach USD 6.3 billion by financial year 2020 (FICCI report 2016). Approximately 50% of the demand comes from domestic consumers while the rest goes towards exports. Consumption of technical grade pesticides in India had a steady growth over the years (Fig. 1). Andhra Pradesh (including Telangana), Maharashtra and Punjab are top three states contributing to 45% of pesticide consumption in India. Pesticides consumption in India is amongst the lowest in the world at 0.6 kg/ha against ~13 kg/ha in China. Pesticide consumption is biased towards insecticides (60% of the pesticide used is insecticide) in India as against 40% globally. Among the crops, cotton and rice consume 57% of the total pesticide consumption. Rice, a prevalent crop in south-east Asia is attacked by number of pests (Fig. 2) due to favourable climatic conditions. 15-25% potential of rice production is lost due



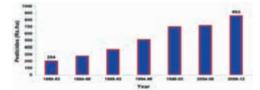


Fig. 1. Pesticide use scenario over the years in value term (The expenditure on pesticide use was Rs.863 at all India level at constant 2013-14 price with highest use in the state of Punjab (Rs.3340/ha) and lowest use in Jharkhand)

different pests, weeds and diseases (Table 1). It compels farmers to use a major chunk of pesticides to prevent/recover from pest attack. In India, Central Insecticide Board and Registration Committee has recommended 90 pesticides or combination product to tackle wide range of pest problems. Most benefits of pesticides are based only on direct crop returns. Pesticide

requirement/demand and import in India is presented in Table 2.

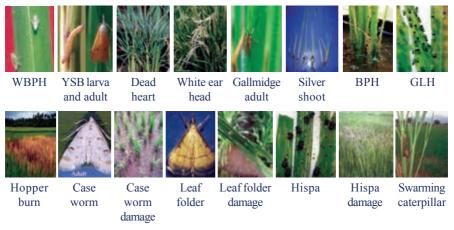


Fig.2. Insect pest in rice and damage symptoms.

## Table 1. Losses due to pest attack.

	Actual production	Approximate estimated lossin yield		Monetary value of estimated losses	
Crop	(milliontonnes)	Percent	In milliontonnes	(millionRs.)	
Cotton	44.03	30	18.9	339660	
Rice	96.7	25	32.2	240138	
Maize	19	20	4.8	29450	
Sugarcane	348.2	20	87.1	70667	
Mustard	5.8	20	1.5	26100	
Groundnut	9.2	15	1.6	25165	
Other oilseeds	14.7	15	2.6	35851	
Pulses	14.8	15	2.6	43551	
Coarse cereals	17.9	10	2.0	11933	
Wheat	78.6	5	4.1	41368	
Total/Average		17.5		863884	

(Dhaliwal et al.2010)



Pesticide demand MT(Tech. Grade)						
				2014-15	2015-16	
2010-11	2011-12	2012-13	2013-14	provisional	projected	
54637	58368	53882	61153	64966	63154	
Pesticide consumption MT(Tech. Grade)						
55540	52979	45619	60282	57353	-	

#### Table 2. Requirement/demand of pesticides and import of pesticides in India.

Source: Standing Committee on Agriculture (2015-2016) Sixteenth Lok Sabha Ministry of Agriculture and Farmers Welfare (Department of Agricultural Research and Education) Twenty Ninth Report

Post harvest losses were estimated from 9 per cent in developed countries to 20 per cent or more in developing countries due to stored product insects. Concepts of "a grain saved is a grain produced" and "hidden harvest" should be an integral part to achieve food security. The most used fumigants are methyl bromide and phosphine. Methyl brome is being phased out by many countries for its ozone-depleting nature. Several reports pointed out that due to repeated use of phosphine led to the development of pest resistance. Lack of new discoveries and strict fumigant registration has added more challenges. There is an urgent need to evaluate and find the most effective dose of fumigant against rice storage pests.

Despite the beneficial effects, there is genuine concern over the use of pesticides and its impact to non-target organisms especially human being. This is because small amounts of pesticide residues may remain in the crops, either resulted from the direct use of pesticides on the crops or environmental contamination. In India problems resulting from unregulated and uncontrolled usage are quite alarming. Over 98% of sprayed insecticides and 95% of herbicides reach a destination other than their target species, because they are sprayed or spread across entire agricultural fields.Runoff and wind may cause non-point pesticide pollution and affecting other species. It is relevant to know the concentration of pesticides presents in different matrices and if there is certain scope to avoid the pesticide contamination.

## 1.1. Objectives

- i. To generate baseline susceptibility data for the newer chemistry molecules against insect pests and diseases.
- ii. To study the mechanism of pesticide resistance and management of resistance.
- iii. 'To investigate long term effect of pesticides on soil flora and fauna
- iv. To check pesticide related food and environmental safety issues

## 2. STATUS OF RESEARCH KNOWLEDGE

#### 2.1. New molecules and assessing their effectiveness against insect-pests

Researchers are working tirelessly to develop safer molecules which could undergo photo-degradation, microbial degradation as well as chemical degradation leaving



very less amount of residues in the environment. Accordingly, many conventional pesticides have been replaced by newer insecticides. These new group of insect control insecticides includes neonicotinoids, spinosyns, avermeetins, oxadiazines, IGR's, fiproles, pyrroles, pyridine azomethine, ketoenols and benzenedicarboxamides. Most of these groups of pesticides play an important role in managing many arthropod pests with good bioefficacy, high selectivity and low mammalian toxicity, which make them attractive replacement for synthetic organic pesticides. These novel groups of pesticides are likely to play an important role in IPM programme in future. Classification and mode of action of new chemistry insecticides is presented in Table 3.

Chemical class	Active ingredients	Mode of action
Avermectins, milbemycins	Abamectin, Emamectin benzoate, Lepimectin, Milbemectin	Glutamate-gated chloride channel allosteric modulators
Spinosyns	Spinetoram, Spinosad	Nicotinic acetylcholine receptor allosteric modulators
Diamides	Chlorantraniliprole, Cyantraniliprole,Flubendiamide	Ryanodine receptor modulators
Formamidines	Amitraz	Octopamine receptor agonists
Neonicotinoides	Acetamiprid, Clothianidin, Dinotefuran, Imidacloprid, Nitenpyram, Thiacloprid, Thiamethoxam	Nicotinic acetylcholine receptor competitive modulators
Oxadiazines	Indoxacarb	Voltage-dependent sodium channel blockers
Phenyl pyrazoles	Ethiprole, Fipronil	GABA-gated chloride channel blockers
Pyridine azomethines	Pymetrozine, Pyrifluquinazon	Chordotonal organ TRPV channel modulators
Tetronic and tetramic acid derivatives	Spirodiclofen, Spiromesifen, Spirotetramat	Inhibitors of acetyl CoA carboxylase

Table 3. Classification and mode of action of new chemistry insecticides as per IRAC (Insecticide Resistance Action Committee) (IRAC2015).

Pesticide mixtures may be more effective against various life stages of arthropod pests The primary benefits of mixed pesticide formulations are decreasing labour cost by reduction of rounds of application, higher mortality of different groups of arthropod pests having separate and distinct feeding habits and delaying resistance development against a particular pesticide by various pests. Additive or synergistic effects of insecticides in mixture with botanicals can be obtained to control insect pests (Table 4). Efficacy of insecticides as seedling root dip for YSB in dry season rice has been worked out as effective and low cost technology (Jena 2004). Chemical control of YSB has also been worked out, particularly, the oviposition deterrent activity. Thus the evolution of materials continued with new chemical families discovered and utilization of them in different formations will offer increased pest protection, reduced persistence and less polution. Pesticide tested at ICAR-NRRI against insect pest are presented in Table 5.



Pests	Chemicals	Authors
Nilaparvatalugens	Buprofezin 23.1% + fipronil3.85% SC	Chakraborty et al. 2017
(Brown planthopper)	)	
Yellow stem borer	Lowest per cent of dead heart recorded in tricyclazole + chlorpyriphos combination and white ear in azoxystrobin + chlorpyriphos combinatio	Neelakanth et al. 2017 n
Yellow stem borer	Least per cent of dead heart (1.7%) in application of tricyclazole + fipronil	Prasannakumar et al. 2011

#### Table 4.Status of insecticide mixtures in controlling insect pests.

#### Table 5. Pesticides tested at ICAR-NRRI.

Pests	Chemicals	Authors
Hispa,leaf folder and yellow stem borer	Phorate, carbofuran, beta cyfluthrin, thiacloprid, phosphamidon, monocrotophos	Rath 2002
Termite, yellow stem borer and Gundhi bug	Carbofuran, fenvalerate	Rath 2005
Termite, yellow stem borer and gundhibug	Pyriphos,cypermethrin, phosphamidon, monocrotophos, thiamethoxam, imidacloprid, endosulfan, carbofuran and phorate	Rath 2006
Yellow stem borer and gundhibug	Flubendiamide + buprofezin, monocrotophos, acephate, flubendiamide,dinotefuron,buprofezin	Rath 2011; Rath 2012
Yellow stem borer and gundhibug	Carbofuran,phorate,cartap,chlorpyriphos and monocrotophos	Rath 2013; Rath 2014
Yellow stem borer and gundhibug	Sulfoxaflor,acaphate, dinotefuron, thiamethoxam, triazophos, buprofezin, imidacloprid and monocrotophos	Rath et al. 2014; Rath et al . 2015
Rice hispa, BPH, ear cutting caterpillar, leaf folder and gundhi bug	Imidacloprid, bifenthrin, thiamothoxam, indoxacarb	Jena and Dani 2011; Jena et al. 2000
Yellow stem borer	Chlorantraniliprole	Jena et al 2001

# 3. NEW MOLECULES AND ASSESSING THEIR EFFECTIVENESS AGAINST DISEASES

Rice diseases cause crop losses about 12.2% of the attainable yield. A wide range of rice diseases affect rice, like blast, sheath blight, bacterial blight, brown spot, false smut and several virus diseases, including rice tungro, are of primary concern. As the major rice diseases are caused by fungus, fungicides are important tool to control rice



diseases.Globally 8.4% of fungicides market share is for rice.The rice fungicides can be broadly classified in two categories viz., seed treating and foliar fungicides. Seed treating fungicides have narrow to moderate spectrum of control. Major advantage of seed treating fungicides is its high level of control at low dose and with low residue. Tricyclazole at 0.2g/kg of seed effectively controlled leaf blast. Foliar fungicides are highly effective in managing foliar diseases and those are grouped as per their mode of action and chemical class.(a) Melanin biosynthesis inhibitors are highly effective against rice blast disease; prevent melanin biosynthesis in appressoria of P. oryzae and penetration to rice plants forming appressoria (e.g. tricyclazole, pyroquilon, chlobenthiazone etc.) or scytalonedehydratase enzymes (carpropamid, dichlocymetetc.).(b) Benzimidazole group fungicide (e.g. carbendazim, thiophanate, thiabendazole etc.) was introduced during 1960s and early 1970s are single site inhibitors of fungal microtubule assembly during mitosis, via tubulin-benzimidazoleinteractions. (c) Triazole fungicides (e.g. propiconazole, tebuconazole, hexaconazole, difenconazole etc.), the largest class are highly systemic with mobility through xylem and are known to have broad spectrum activity against major diseases like sheath blight, sheath rot, grain discoloration etc. (d) MET II inhibitors (eg. thifluzamide and flutalonil etc.) inhibit succinate dehydrogenase in fungi and highly effective against sheath blight. These fungicides are systemic (Xylem mobile) and have good residue. (e) Strobilurins, first synthetic group fungicides originally derived from mushroom fungi, called Strobilurustenacellus. These fungicides are referred to as QoI fungicides (Vincelli2002). Some of the other commonly used strobilurins against rice diseases are fenamidone, kresoxim methyl, pyraclostrobin and trifloxystrobin either as stand-alone or mixed with other multi-site inhibitor fungicides or triazoles like propiconazole.

As per Central Insecticide Board, Govt. of India, more than 30 fungicides have been registered for use in rice and several new molecules are under testing. Isoprothiolene and Tricyc1azole 75WP were more effective in controlling the blast disease in nursery in comparision to Isoprothiolene, Tricyclazole, Edifenphos, Hexaconazole and Mancozeb as seed treatment.In rice, strobilurin fungicide trifloxystrobin in combination with tebuconazole are used against blast, sheath blight and other foliar diseases (Bag et al. 2016). Tricyclazole and isoprothiolane are found highly effective resulting in 87.9 and 83.8% reduction in neck blast and 33.8 and 29.9% increase in grain yield over check, respectively (Sachin and Rana 2011).

Optimum rate of azoxystrobin @ 125 g/ha are highly effective. Biswas and Bag (2010) reported new QoI fungicides Kresoxim methyl, azoxystrobin, metaminostrobinand trifloxystrobinand combinations with other groups were highly effective against sheath blight of rice. Copper hydroxide fungicides reduced false smut balls in harvested rice by 80% but yield was also often reduced significantly while Bag et al (2010) reported effectiveness of new formulation of copper hydroxide. Application of Metaminostrobin 20% SC + hexaconazole 5% SC was effective against leaf blast and neck blast. Different pesticide tested against rice diseases are presented in Table 6.



S.No.	Diseases	Fungicides	References
1	Blast	Tricyclazole and Isoprothiolane	Sachin and Rana (2011)
		Kresoxim methyl,metaminostrobin and trifloxystrobin	PrasannaKumar et al. (2011)
		Tricyclazole and azoxystrobin	Kunova et al. 2013
		Tetrachlorophthalide 30 WP, tebuconazole + trifloxystobin and difenoconazole	Ghazanfaret al. 2009
		Azoxystrobin and kresoximmethyl	Chen et al.2015
		Propiconazole	Fang et al. 2009
2	Sheath blight	Azoxystrobin and propiconazole Kresoxim methyl,metaminostrobin and trifloxystrobin	Parsons et al.2009 PrasannaKumar et al.2011
		Thifluzamide Trifloxystrobin 25% + tebuconazole50%	PrasannaKumar et. al. 2012 Bag2009
3	Brown spot	Captan 70% + hexaconazole5% WP @ 0.2%	Kiranand Prasanna 2011
4	False smut	Zineb and thiophanatemethyl Trifloxistrobin + tebuconazole	Kannahi et al. 2016 Raji et al. 2016
		Copper hydroxide	Bag et al. 2010
5	Bakanae/ Foot rot	Carbendazim	Bagga et al. 2006
6	Seedling blight	Carbendazim 12% + mancozeb63%, trifloxystrobin50% + tebuconazole25%.	Raghu et al. 2017

#### Table 6. Literature on efficacy of fungicides in managing rice diseases.

## 4. PESTICIDE RESISTANCE-ITS CAUSE, HOW TO OVERCOME IT

Pesticide resistance is a reduction in the ability of an insecticide in achieving the desired control. This is reflected in repeated failure of a pesticide expected level of control of pests when used according to the product label recommendations. When a pesticide is first used, a small proportion of the pest population may survive exposure to the material due to their distinct genetic makeup. These individuals pass along the genes for resistance to the next generation. Subsequent uses of the pesticide increase the proportion of less-susceptible individuals in the population. Through this process of selection, the population gradually develops resistance to the pesticide. It may be behavioral, penetration, metabolic or/and altered target-site resistance. In addition, failure to adhere to good farming practice such as crop rotation and cleaning of farm equipment, which helps prevent the spread of pest seeds and spores, can exacerbate the spread of resistance. Fungicides having single site action are more prone to develop resistant mechanisms in the pathogen compared to those having multi cite action. Status of insecticide resistance in India and world is given in Table 7. Thus, industry has given emphasis in the research particularly areas of mode of action. resistance risk, field monitoring for baseline sensitivity and sensitivity variations in treated fields.



#### Table 7.Status of insecticide resistance in India and world.

S. No	. Resistance status	Authors
1	In China field-collected populations of <i>Nilaparvatalugens</i> had developed high levels of resistance to imidacloprid (resistant ratio, RR = 233.3–2029-fold) and buprofezin (RR = 147.0–1222). Furthermore, <i>N. lugens</i> showed moderat to high levels of resistance to thiamethoxam (RR = 25.9–159. and low to moderate levels of resistance to dinotefuran (RR = 6.4–29.1), clothianidin (RR = 6.1–33.6), ethiprole (RR = 11.5–71.8), isoprocarb (RR = 17.1–70.2), and chlorpy (RR = 7.4–30.7).	2)
2	Most strains of <i>N. lugens</i> (except FQ15) collected in 2015 had developed moderate resistance to dinotefuran, with resistance ratios (RR) ranging from 23.1 to 100.0 folds.	Mu et al. 2016
3	Field populations collected from different locations of Karnataka (Gangavati, Kathalagere, Kollegala, Soraba and Mandya) were studied for their susceptibility or resistance to the insecticides and found that the populations from Gangavati, Kathalagere and Kollegala exhibited higher resistance to some of the old insecticides and low resistance to new molecules.	Basanth et al. 2013
4	Brown plant hopper population collected from east Godavari district of Andhra Pradesh exhibited 5- to 35-fold resistance to neonicotinoid insecticides like imidacloprid, thiamethoxam and clothianidin.	Krishnaiah et al. 2006
5	Moderate levels of resistance were detected in the field populations to acephate, thiamethoxam and buprofezin (resistance factors 1.05–20.92 fold, 4.52–14.99 fold, and 1.00–18.09 fold, respectively)	Malathi et al. 2017

#### 4.1. Major factors that influence resistance development

- Continued and frequent use of one pesticide or closely related pesticides on a insect pest population
- Use of application rates that are below or above those recommended on the label
- Poor coverage of the area being treated
- Frequent treatment of organisms with large populations and short generation times
- Failure to incorporate non-pesticidal control practices when possible
- Simultaneous treatment of larval and adult stages with single or related compounds.

#### 4.2. Steps to be taken to overcome it

The best strategy to avoid insecticide resistance is prevention. More and more pest management specialists recommend insecticide resistance management programs



as one part of a larger integrated pest management (IPM) approach. Monitoring is one of the key activities in the implementation of an insecticide resistance management strategy. Monitoring insect population development in fields to determine when control measures are warranted. Monitor and consider natural enemies when making control decisions in some cases. Insecticides should be used only if insects are numerous enough to cause economic losses that exceed the cost of the insecticide plus application. It is better to have an integrated approach to managing pests.Use as many different control measures as possible. Avoid broad-spectrum insecticides when a narrow-spectrum or more specific insecticide will work. Apply insecticides when the pests are most vulnerable. Use application rates and intervals recommended by the manufacturer Thus in nutshell the successful management of insecticide resistance requires monitoring the levels of resistance and understanding the mechanisms involved. Such studies are necessary to enhance the control efficiency by alternating appropriate insecticides.

### 5. UNDERSTANDING ROLE OF PHOSPHINE IN RICE STORAGE PESTS MANAGEMENT

Phosphine has been in commercial use as a grain fumigant since the mid 1950s. However, Phosphine (from aluminium phosphide tablet and powder formulations) is the only fumigant used for grain protection in India since 1970s. More recently its use has expanded due to the phase-out of methyl bromide as they create ozone depletion. Multiple phosphine applications (every 3 months during a storage period of 1 to 3 years) of grain stacks are common. Inadequacies in current fumigations such as use of substandard gas proof sheets and sand stacks, shorter exposure periods (3 to 5 days), failure to measure gas levels, and poor maintenance of gas concentrations during exposure period are major constraints of successful fumigation.

Phosphine fumigation is an effective method of eliminating insects in stored commodities in many countries worldwide. It should be noted that there is little to be gained by extending the exposure period if the structure to be fumigated has not been carefully sealed and insects are not subjected to lethal concentration of phosphine. It has been found that as regards technical performance, Quickphos (phosphine source), when applied either in single or double dosage, exhibited in the control of stored-product insects such as *Rhyzopertha dominica*, *Sitophilus* spp., and *Tribolium castaneum*, yielding 100% mortality.Toxic hydrides produce by phosphine cause changes to cellular and organismal physiology, including disruption of the sympathetic nervous system, suppressed energy metabolism and toxic changes to the redox state of the cell. It was recommended that at 1.0, 0.3, and 0.2 mg l<sup>-1</sup> complete control can be expected in 5, 10 and 14 days, respectively for field trial and eventual registration.

The main disadvantages of phosphine fumigant are that the treatment confers no residual protection against re-infestation, once the commodity is again exposed, and the fact that the most effective fumigants are all highly toxic to humans and other non-target organisms. There is no doubt that good fumigation practices also prevent



insect survival, which is assumed as preventing further insect resistance. Phosphine resistance in grain beetle pests particularly in *Rhyzopertha dominica*, *Tribolium castaneum*, *Cryptolestes* spp., has been elaborated. To prevent the development of resistance, it is essential to avoid applications with sub-lethal doses. Depending on fumigation circumstances, in particular low temperature and poor gas-tightness of the container, it is important to use longer exposure to achieve pest mortality in all parts of the fumigated commodities. In addition it is necessary to achieve a minimum of 500 ppm for the control of normal insects and at least 1000 ppm when phosphine-resistant insects are present as target end concentrations.

## 6. EFFECT OF PESTICIDES ON SOIL MICRO FLORA AND FAUNA

In the present situation, pesticides application in agriculture becomes a necessary evil which resulted in contamination of aquatic and soil ecosystems and thus affected the microbial community inhabit of those ecosystems. Microbial communities are one of the key drivers of assessing soil health and therefore for the advancement of sustainable agriculture a proper understanding is to be required to visualize the changes of soil microflora change under influence of chemical pesticides. Application of higher dose of chemicals and fertilizers in agriculture actually warrants us to know the real effect on soil microflora, but complete data are not available to justify the actual impact of these on soil microbial communities. Besides, there are still multiple issues which need to be addressed to estimate the effect of pesticides on microbial communities in the soil in the future, and to make a broadly accepted agenda for risk assessment in agro-ecosystems that include microbial indicators.

According to guidelines for the approval of pesticides, carbon or nitrogen mineralization is the most important functional parameters to judge the side-effects of pesticides on soil microorganisms under any systems (Kumar et al. 2017a). Some microbial groups use applied pesticides as a source of energy and nutrients, whereas other groups may be affected by toxic nature of the pesticides. A variation of soil microbial community under influence of pesticides is a complex phenomenon and thus provides an insight of two important implications of microbial diversity. Firstly, a decrease in diversity must have resulted in the risk of alteration of their biological response in a particular system. Secondly, alteration of microbial diversity itself provides information about the intensity of such stressed ecosystem. Therefore, it is necessary to be examined the non-target effect of pesticide on soil microflora and its diversity under a particular system. Thus, we need to have a wider method to enumerate soil microflora under pesticide exposure, and usage of latest molecular tools such as qPCR and metagenome for better understanding the abundance and diversity of soil microbial community under influence of long-term exposure of pesticides in agricultural crops including rice.

In the past, a different array of cultivation-dependent and independent methods were used to analyze the effects of pesticide exposure on soil microflora in the different



ecosystem, however, under rice soils, it has been nominally investigated (Kumar et al.2017b; Kumar et al. 2017c). Among all pesticides, 82% of the data refer to insecticides and on an average, pesticide exposure resulted in the increased and decreased of bacterial population by 17% and 25%, respectively, whereas, 58% of the cases no significant change was noticed. The same trend continued to the actinomycetes population, whereas results indicated that fungal groups were found to be most sensitive to pesticides. Some reports also indicated that among the different groups, nitrogen mineralization bacteria (ammonium oxidizers, denitrifiers, and nitrite oxidizers) were seemed to be the negatively affected by the continuous application of chlorpyrifos (insecticide) (Kumar et al. 2017a), while other bacteria were relatively less frequently inhibited. The absence of inhibitory effect on populations of diazotrophs is in agreement with a very low record of negative effects of pesticide application on nitrogen fixation in soil (Table 8).

Population/Activity		Pesticide tested*			Effect**		
MICROBIAL POPULATIONS	F	Η	Ι			+	
Actinomycetes	0	5	26	4	19	7	
Fungi	0	1	25	7	18	1	
Bacteria							
Total bacteria in soil	0	5	15	4	13	3	
Total bacteria in Phyllosphere	0	0	7	0	7	0	
Total bacteria in rhizosphere	0	0	7	0	7	0	
N cycle other than BNF	0	9	5	6	3	5	
N <sub>2</sub> -fixing bacteria	0	1	20	1	15	6	
Various Physiological groups	0	5	5	1	2	7	
Miscellaneous groups	0	2	4	3	3	1	
Total of Bacterial counts	0	22	63	15	50	22	
SoilProperties(N,P,Kavailability)	3	7	0	1	0	8	
Specific Enzymatic Activities							
Amylase	0	1	8	0	8	1	
Cellulase	0	0	8	0	8	0	
Dehydrogenase	1	4	3	0	6	3	
Dextranase	0	0	7	0	7	0	
Invertase	0	1	14	0	15	0	
Phosphatase	0	0	13	0	13	0	
Urease	0	4	1	0	4	1	
â-glucosidase	0	0	13	4	9	0	
Others	0	0	2	0	2	0	
Total of enzymatic activities	1	10	69	4	72	5	

## Table 8. Summarization of the data from published reports on the effects of pesticides on microflora and microbial activities in wetland rice fields (Source: Dey 2012).

Contd....



Population/Activity		Pesticide tested*			Effect**		
MICROBIAL POPULATIONS		Н	Ι				
Microbiological activities							
O <sub>2</sub> uptake or CO <sub>2</sub> Production	0	5	2	2	1	3	
OMdecomposition/mineralization	1	2	1	0	4	0	
Nitrification	2	6	15	14	6	2	
Denitrification	9	9	12	4	16	0	
N <sub>2</sub> -fixation (soil)	8	9	24	1	3	28	
N <sub>2</sub> -fixation (rhizosphere)	0	0	32	13	10	9	
Total microbiological activities	20	31	86	34	40	42	
Grand total	24	76	269	65	199	85	

I: insecticide; H: herbicide; F: fungicide; \*Summary per microbiological groups and microbial activities; \*\* - inhibition, =: no effect, +: enhancement

## 7. EFFECT OF CHEMICALS ON THE ABUNDANCE AND DIVERSITY OF SOIL ARTHROPODS IN RICE ECOSYSTEM

Though not apparent to the naked eye, soil is actually one of the most diverse and species rich habitats of the terrestrial ecosystem. The total number of described species on earth (1,500,000) and 23 per cent are soil animals. Historically, most of the efforts on biodiversity studies focused, especially on above ground plant and animal species. However, the below ground biota supports much greater diversity of organisms than does the above ground biota. The under agro-ecosystem, earthworms were the most dominant organism in terms of biomass, while in terms of numbers, ants and termites predominated.External agricultural inputs such as mineral fertilizers, organic amendments, herbicides, fungicides and pesticides are applied with the ultimate goal of maximizing productivity and economic returns, while side effects on soil organisms are often neglected. Pesticides and fertilizers are integral part of agriculture and studies related to their impact are well documented. Pesticides like Aldrin and DDT, metal pollutants, Zn have adverse impact on soil fauna. Chlorpyriphos application adversely affected beneficial arthropods like non-Sminthurid Collembolans, ants, spiders and parasitic hymenoptera. Fertilizer application, pre emergence and post emergence herbicides had some negative impact on the faunal activity. In Kentucky blue grass turf, chlorpyriphos and isofenphos had the greatest impact on predacious arthropods.

# 8. PESTICIDES RESIDUES IN SOIL-PLANT-WATER SYSTEM

Upon application, pesticides undergo a very complex series of events. It may reach to target site to kill the organisms or it may be transported into environmental matrices through the air or water. Sometimes it may reach into the ground. Distribution of pesticides depends on its nature and pertaining environment. It has been observed that there is a significant knowledge gap about movement of pesticide and its fate in



the environment. Proper pesticide residue analysis across the globe in a network will help to minimize the pesticide pollution. Every steps to be taken to release minimum quantity of pesticides to save our environment.

Despite the health risks from pesticides, farmers believe it is indispensable for higher production. It has been observed at farm level improper use of pesticides has further contributed to the environmental and health problems resulting from pesticides. Improper uses may be in the form high dosages, use of non-recommended pesticides, inadequate pre-harvest intervals and cocktailing of pesticides. Untrained pesticide shopkeepers play a critical role for improper and more use of pesticides. Most of developing countries are unsuccessful to regulate the pesticide use and its market despite its stringent laws.

Pesticides sprayed in field have a less chance to be quantified in rice grains. Dehusking and milling can remove residues at various extent as pesticides are mostly contained in outer layer of grain i.e. bran. Pesticides are lipophilic in nature and there is a greater chance they are contained in rice bran. There are very few pesticides can translocate into the flour. But during grain storage, rice is invariably sprayed with insecticides to reduce losses. This leads to pesticide contamination in food. In rice ecosystem, large amount of standing water creates the probable problems of pesticides contamination in ground and surface water. Leaching or runoff depends not only on soil properties (clay content, organic matter content etc.) but also on pesticides properties like solubility, residual half-life, etc. However, to maximize the benefits of pesticide use at minimum human, environmental and economic cost, pesticides must be strictly regulated and used judiciously by properly trained and appropriately equipped personnel, ideally in tight integration with other complementary technologies.

Continuous application of chlorpyrifos for 7 years did not affect most of soil microbiota except nitrogen mineralizing microflora (Kumar et al. 2017a). Chlorpyriphos degradation was faster under elevated  $CO_2$  (Adak et al. 2016). Changes in microbial diversity indices confirmed that imidacloprid application significantly affected distribution of microbes. The extent of negative effect of imidacloprid depends on dose and exposure time (Mahapatra et al. 2017). Pretilachlor did not harm the soil microbes at field dose but affected at higher dose (Sahoo et al. 2016). In-vitro experiment has been carried out for number of pesticides namely butachlor, bispyribacsodium, chlorantraniliprole, fipronil etc. to check their distribution in different environmental matrices and effects on soil microbes.

#### 9. KNOWLEDGE GAPS

In India, work on pesticide resistance and its management have not been given much emphasis compared to developed countries. Recent reports of pesticide resistance should be deeply understood to overcome the problem. Consumers are concerned about the pesticide residue on their food. Simple but exhaustive analytical method should be developed to quantify minimum quantity of pesticides. Short term



studies of pesticide poisoning were reported elsewhere. Our study on long term effect of pesticide will provide inputs on structural and functional changes of soil flora and fauna upon pesticide application.

### **10. RESEARCH AND DEVELOPMENT NEEDS**

Based on the above observation, generation of baseline information of newer chemicals about their effectiveness and variation in location should be investigated. In addition to that, pesticide mixtures should be tried to overcome the resistance problems. The mechanism of insecticide resistance should be studied for future research. Impact of long-term pesticides on rice insect pests, soil fauna, microbes and AM fungal associations in rice-rice cropping system should be determined. Loads of pesticides in soil- plant- water system should be quantified to make a rice cropping system more sustainable and eco-friendly.

#### **11. WAY FORWARD**

#### 11.1. Managing pesticide resistance

The main purpose of resistance management is to prevent or at least slow down the accumulation of resistant individuals in insect pest populations, so as to preserve the effectiveness of available pesticides. The challenge is to reduce the selection pressure for resistance while providing the necessary level of crop protection. There is unfortunately no single resistance management prescription that can be applied globally to all pesticides, insect pests and crops. Nor is resistance solely a technical problem that can be readily overcome with the right new pesticide with a new mode of action, or an adjustment in the way conventional pesticides are used. Managing resistance requires: first, the use of rational pest control strategies based on the principles of integrated pest management, which reduce pesticide use and hence the selection pressure for resistance; and second, the implementation of a comprehensive and tailor-made Resistance Management Plan (RMP) that is adapted to the pest, the crop and the region.

#### 11.2. Alternative of phosphine fumigations

Alternative to phosphine such as ethylformate, sulfurylfluoride and  $CO_2$ -rich atmosphere have been studied both at laboratory and field levels and efficacy proven. However, these have not been used yet for grain preservation in India. Several plant compounds have been studied but at laboratory level only. Overall, while there is an appreciable change in Indian grain storage system by the use of silo bags substituting CAP storage and by expanding storage capacity by erecting more metal silos across the county, rigorous changes in fumigation of food grains are yet take place.

#### 11.3. Cheaper methods to detect pesticide residue

Till date pesticides have been quantified through chromatographic methods coupled to selective detectors, for example, GC-MS, LC-MS-MS. These methods are efficient, sensitive and reliable. Major limitation of these techniques is time-consuming



and costly and need trained technicians. Cheap and easy methods which can reliably detect pesticides in different food products into the homes have to be developed. Considerable attention has been given to the development of biosensors for the detection of pesticides as a promising alternative. Ready to use device like Electronic-nose (e-nose) methods should be tested for rapid detection of pesticides. This will be at low cost of detection. Scientists already developed an electronic nose gas-sensing device. It was based on intrinsically conducting polymer (CP)-type. This device could identify eleven insecticides representing eight different classes as well as can discriminate them. Steps have been taken to have in-build library into the e-nose based on electronic vapor signature patterns.

## 11.4. Moving towards greener chemicals and green practices in pesticide usage

In recent years, neonicotinoids and diamides have been the fastest-growing class of insecticides in modern crop protection, with widespread use against a broad spectrum of sucking and certain chewing pests. This provides room for more innovative technology to be developed in application of newer molecule pesticides. Of such the technologies are 1) Employing pesticides as seed treatment to provide protection to seedlings against insect pests, and 2) Using insecticides mixtures having independent mode of action. Dermacor-X-100® (active ingredient, chlorantraniliprole) seed treatment could be used as a valuable component of integrated pest management program for stem borers in rice. Research for refined use of seed treatments is anticipated. Status of insecticide seed treatment in controlling stem borers is presented in Table 9.

Pests	Chemicals	Authors
Chilopartellus and Sesamiainferens	Chlorpyriphos 20 EC (Seed treatment- ST + Foliar spray-FS) was found best among all the treatments	Hedge et al. 2017
Stem borer complex in rice in Texas ( <i>Eoreumaloftini</i> and <i>D. saccharalis</i> )	Dermacor-X-100 <sup>®</sup> (0.1 mg a.i per seed) seed treatment provided complete control.	Way et al. 2009
Chilopartellus	Seed treatment with Spinosad 45%SC spray @ 200ml/ha	Vishvendra et al. 2017

## **12. CONCLUSIONS**

Proper pest monitoring, protective clothing, application of appropriate pesticide at right time at right dose and target species should be integral part of pesticide application. Genuine concerns on consumer and environmental safety of pesticide uses should be dealt with scientific findings. Need of the hour is to have a readymade pesticide detection kit at affordable price. Long term pesticide uses and its effect on flora and fauna should be investigated. Mass awareness among end users about optimization of chemical pesticide use in rice is the need of the hour.



#### References

- Adak T, Munda S, Kumar U, Berliner J, Pokhare SS, Jambhulkar NN and Jena M (2016) Effect of elevated CO<sub>2</sub> on chlorpyriphos degradation and soil microbial activities in tropical rice soil. Environmental Monitoring and Assessment 188:105.
- Bag M K (2009) Efficacy of a new fungicide 'Trifloxystrobin 25% + Tebuconazole 50%' 75WG against Sheath Blight (*Rhizoctonia solani* Kühn) of Rice. Journal of Crop and Weed 5(1):224-226.
- Bag MK, Saha S and Rai RK (2010) Fungitoxicity of a new formulation of copper hydroxide (Kocide 2000 54 DF) against false smut disease of rice in West Bengal. Pestology 34(1):26-28.
- Bag MK, Yadav M and Mukherjee AK (2016) Bioefficacy of Strobilurin Based Fungicides against Rice Sheath Blight Disease. Transcriptomics 4:128.
- Bagga PS and Kaur S (2006) Evaluation of fungicides for controlling false smut (*Ustilaginoidea virens*) of rice. Indian Phytopathology 59(1):115-117.
- Basanth YS, Sannaveerappanavar VT and SiddeGowda DK (2013) Susceptibility of different populations of *Nilaparvata lugens* from major rice growing areas of Karnataka, India to different groups of insecticides. Rice Science 20(5): 371"378.
- Biswas A and Bag MK (2010) Strobilurins in management of sheath blight disease of rice: A review. Pestology 34(4): 23-26.
- Chakraborty G, Roy D and Mondal S (2017) Ready-mix formulation of buprofezin 23.1%+fipronil 3.85% SC for eco-friendly and economic management of brown plant hopper in rice.Research on Crops18(2):364-369.
- ChenY, Yang X, Yuan SK, Li YF, Zhang AF, Yao J and Gao TC (2015) Effect of azoxystrobin and kresoxim-methyl on rice blast and rice grain yield in China. Annals of Applied Biology 166(3): 434–443
- Dey A (2012) Population dynamics & characterization of selected plant growth promoting rhizobacteria (PGPR) frompesticide treated paddy soil. MSc dissertation. Department of Microbiology, OUAT, Orissa, pp 1-44.
- Dhaliwal GS, Jindal V and Dhawan AK (2010) Insect pest problems and crop losses: changing trends. Indian Journal of Ecology 37(1): 1-7.
- Fang M, Yan L, Wang Z, Zhang D and Ma Z (2009) Sensitivity of *Magnaporthe grisea* to the sterol demethylation inhibitor fungicide propiconazole. Journal of Phytopathology 157:568–572.
- Ghazanfar MU, Waqas Wakil, ST Sahi and Saleem-il-Yasin (2009) Influence of various fungicides on the management of rice blast disease. Mycopathologia 7(1):29-34.
- Hegde K, Manjunatha M, Sharanabasappa, Kalleshwaraswamy CM and Adarsha SK (2017) Effect of Application of biopesticides and insecticides on stem borers and yield of maize (*Zea mays L.*) International Journal of Pure & Applied Bioscience 5(1):42-47.
- Jena M (2004) Efficacy of new insecticides as seedling root dip treatment against yellow stem borer in *Rabi* rice. Indian Journal of Plant Protection 32(2):37-39.
- Jena M and Dani RC (2011) Evaluation of insecticides against rice hispa, *Dicladispa armigera* Oliver (Coleoptera:Chrysomelidae). Oryza 48 (3):255-257.
- Jena M, Dani RC and Rajamani S (2000) Efficacy of new insecticides against rice brown planthopper, Nilaparvata lugens Stal. Oryza 37(3):262-264.



- Jena M (2001) Chemical control of yellow stem borer, *Scirpophagaincertulas* Walker in rabi rice. Indian Journal of Plant Protection 29(1&2):43-46.
- Kannahi M, Dhivya S and Senthilkumar R (2016) Biological control on rice false smut disease using *Trichoderma* species International Journal of Pure & Applied Bioscience 4(2):311-316.
- KiranKumar N and PrasannaKumar MK (2011) Potentiality of new fungicide formulation captan 70% + hexaconazole 5% WP against sheath blight and brown leaf spot of rice. In: Nat. Symposium "Integrated disease management strategies in relation to climate change in South India" IPS (south zone), India p. 27
- Krishnaiah NV, Pasalu IC and Jhansilakshmi V (2006) Neonicotinoid insecticide resistance in agricultural pests. DRR Technical Bulletin 19:48.
- Kumar U, Berliner J, Adak T, Rath PC, Dey A, Pokhare SS, Jambhulkar NN, Panneerselvam P, Kumar A, Mohapatra SD (2017a) Non-target effect of continuous application of chlorpyrifos on soil microbes, nematodes and its persistence under sub-humid tropical rice-rice cropping system. Ecotoxicology and Environmental Safety 135:225-235.
- Kumar U, Panneerselvam P, Govindasamy V, Vithalkumar L, Senthilkumar M, Banik A, Annapurna K (2017b) Long-term aromatic rice cultivation effect on frequency and diversity of diazotrophs in its rhizosphere. Ecological Engineering 101:227-236.
- Kumar U, Shahid M, Tripathi R, Mohanty S, Kumar A, Bhattacharyya P, Lal B, Gautam P, Raja R, Panda BB, Jambhulakar NN, Shukla AK and Nayak AK (2017c) Variation of functional diversity of soil microbial community in sub-humid tropical rice-rice cropping system under long-term organic and inorganic fertilization. Ecological Indicators 73:536-543.
- Kunova A, Pizzatti C and Cortesi P (2013).Impact of tricyclazole and azoxystrobin on growth, sporulation and secondary infection of the rice blast fungus, *Magnaporthe oryzae*. Pest Management Science 69(2):278-284.
- Mahapatra B, Adak T, Patil NKB, BasanaGowda G, Jambhulkar NN, Yadav MK, Panneerselvam P, Kumar U, Munda S and Jena M (2017) Imidacloprid application changes microbial dynamics and enzymes in rice soil. Ecotoxicology and Environmental Safety 144: 123-130.
- Malathi VM, Jalali SK, SiddeGowda SK, Mohan M and Venkatesan T (2017) Establishing the role of detoxifying enzymes in field-evolved resistance to various insecticides in the brown planthopper (*Nilaparvata lugens*) in South India. Insect Science 24: 35–46.
- Mu X, Zhang W, Wang L, Zhang S, Zhang K, Gao C and Wu S (2016). Resistance monitoring and cross-resistance patterns of three rice planthoppers, *Nilaparvata lugens, Sogatella furcifera* and *Laodelphax striatellus* to dinotefuran in China. Pesticide Biochemistry and Physiology 134:8–13.
- Neelakanth, SiddeGowda DK, Basavaraju BS, Shivashankar T and Yereshimi R (2017) Efficacy of insecticide and fungicide combinations against Rice leaf folder and yellow stem borer in field condition. Journal of Entomology and Zoology Studies 5(4):126-128.
- Parsons CE, Robinson JC, Yingling JA and Cartwright RD (2009) Effectiveness of New Foliar Fungicides to Control Sheath Blight of Rice.AAES Research Series 581.
- PrasannaKumar MK, SiddeGowda DK, PandurangeGowda KT, Arpita KS and Ganesh Bhat (2011) Compatibility and efficacy of insecticides and fungicide mixtures against major pests and diseases in rice. Pestology (5):17-21.
- PrasannaKumar MK, Siddegowda DK, PandurangeGowda KT and Vishwanath K (2012) A new carboxnilide group fungicide against paddy sheath blight. Research Journal of Agricultural Sciences 3(2):500-505.



- Raghu S, Lenka S, Adak T, Mukherjee AK and Jena M (2017) Seedling blight problem in upland ecosystem. NRRI News Letter 38(1):12.
- Raji P, Sumiya KV, Renjisha K, Dhanya S and Narayanankutty MC (2016) Evaluation Of fungicides against false smut of rice caused by *Ustilaginoidea virens*. International Journal of Applied and Natural Sciences 5:77-82.
- Rath PC, Chakraborty K, Nandi P and Moitra MN (2015) Field efficacy of some new insecticides against rice stem borer and gundhi bug in irrigated rice ecology. International Journal of Plant, Animal and Environmental Science 5(2):94-96.
- Rath PC (2002) Evaluation of insecticides against insect pests of rice in Assam. Oryza 39(1&2):67-69.
- Rath PC (2005) Efficacy of granular and botanical pesticide against insect pest of upland rice at Hazaribagh. Journal of Plant Protection and Environment 2(1):124-126.
- Rath PC (2011) Testing of some new insecticides against insect pest of rice. Journal of Plant Protection and Environment 8(1):31-33
- Rath PC (2012) Field evaluation of newer insecticides against insect pests of rice. Indian Journal of Plant protection 40(2):148-149
- Rath PC(2013). Field efficacy of granular insecticides against yellow stem borer and gundhibug of rice. Oryza 50(2):194-195.
- Rath PC (2014) Field efficacy of granular insecticides against rice gundhi bug and yellow stem borer. Indian Journal of Plant Protection 42(3):208-210.
- Rath PC, Lenka S, Mahapatra SD and Jena M (2014) Field evaluation of selected insecticides against insect pests of wet season transplanted rice. Oryza 51(4):324-326.
- Rath PC (2006). Efficacy of insecticides against insect pest of upland rice. Journal of Plant Protection and Environment 3(1):115-117
- Sachin U and Rana SK (2011) Effect of fungicides on neck blast incidence and grain yield of rice in mid hills of Himachal Pradesh, Plant Disease Research 26(2): 196.
- Sahoo S, Adak T, Bagchi TB, Kumar U, Munda S, Saha S, Berliner J, Jena M and Mishra BB (2016) Non-target effects of pretilachlor on microbial properties in tropical rice soil. Environmental Science and Pollution Research 23(8):7595-602. (doi 10.1007/s11356-015-6026-x).
- Vincelli P (2002) QoI (Strobilurin) Fungicides: benefits and risks. The Plant Health Instructor. DOI: 10.1094/PHI-I-2002-0809-02.
- VishvendraKumar S, Vaibhav V, Kumar A and Singh DV (2017) Efficacy of different management practices against Chilopartellus (Swinhoe) in *Kharif* maize crop in western Uttar Pradesh, India. International Journal of Current Microbiology and Applied Sciences 6(10):2870-2874.
- Way MO, Nunez MS, and Pearson RA (2009) Insecticidal seed treatments and conservation tillage. Proc. 12th Ann. National Conservation Systems Cotton & Rice Conf. Cotton Incorporated.
- Zhang X, Liao X, Mao K, Zhang K, Wan H. and Li J (2016).Insecticide resistance monitoring and correlation analysis of insecticides in field populations of the brown planthopper *Nilaparvata lugens* (stål) in China 2012–2014. Pesticide Biochemistry and Physiology 132:13-20.\*



## Improving Protein Content, Glycemic Index, Mineral Bioavailability and Antioxidant Value of Rice

#### A Kumar, SG Sharma, N Basak, G Kumar, LK Bose and N Umakanta

#### **SUMMARY**

Rice grain quality is a multifaceted trait and is the prime determinant of consumer choice and marketability of a variety. Thus, improving nutritional quality occupies an important position in rice improvement programs. Climate change and its associated consequences further emphasise upon development of nutrient packed rice grains to cater to the nutritional needs of the millions of people, who are predominantly dependent on rice. Currently available rice varieties are poor in grain protein content which affects the health of rice eating population. Hence, enhancement of total protein in rice grain along with increase in the amount of essential amino acids such as lysine and threonine is of immense importance. Recent research at NRRI has led to release of high protein rice varieties. Though milled rice is normally consumed, brown rice is nutritionally superior as it is richer in nutrients including minerals. Unfortunately, milling results in further reduction in the nutritional quality of rice. In addition, rice contains phytic acid (PA), an anti-nutrient, which further restricts the bioavailability of divalent cations. Thus, enrichment of mineral profile with reduction in PA content is also a research priority. As rice is a carbohydrate rich grain, its glycemic index (GI) is generally high, which makes it unsuitable for consumption by the diabetics. Hence, research on low GI rice is a priority and has resulted in identification of low GI rice and an insight into the factors that determine GI. The adverse effect of the changing climate has seriously affected human health also due to an increase in production of pollutants that tend to enhance the amount of reactive oxygen species. In recent times, pigmented rice have received increased attention from consumers for their inherently high content of bioactive compounds which provides antioxidative, antiinflammatory and other health benefits. Several pigmented rice with high anthocyanin content have been identified. Recently, a cross between a pigmented rice and a white rice has resulted in grains with an almost black endosperm at NRRI.

In view of the changing life style and global climate, it is imperative to identify and develop high yielding rice varieties with better nutritional profile that suit even diabetics and also ensure better availability of nutrients including minerals and antioxidants.

#### **1. INTRODUCTION**

Rice, a dietary staple for half of the world's population, accounts for 27% of dietary energy supply, 20% of dietary protein and 3% of dietary fat. Removal of the inedible husk of the paddy grain results in brown/unprocessed rice which is



nutritionally rich but has shorter shelf life. As a result, milling is carried out to remove the aleurone layer. This leaves the starchy grain deficient in many nutrients including protein, oil and micronutrients (vitamins and trace minerals) which are further lost during washing and cooking. Thus identification and development of nutritionally superior rice is a research priority to popularize consumption of rice varieties with high grain protein content, mineral bioavailability and high antioxidant value. This becomes more important in the context of changing climate, which demands that the masses consume nutrient dense food. The objective of the present chapter is to elaborate on the nutritional quality of rice particularly in relation to protein content, glycemic index, mineral bioavailability and antioxidant value.

### 2. HIGH PROTEIN RICE

Milled rice is low (6-7%) in protein content (Juliano 1964). Over the past decades, improvement in protein content of milled rice has been a key nutritional target in breeding programs. The nutritional status of a variety is mainly dependent on its protein content. Rice protein, when compared to that of other grains, is considered one of the highest quality proteins. It has all eight of the essential amino acids with a biological value of 86 (the BV of 70 or above indicates acceptable quality). Though, it is a good source of aspartic and glutamic acids, it provides insufficient amounts of lysine and threonine resulting in serious malnutrition.

The enrichment of rice grains with protein would have a positive effect on the health of billions of people around the globe particularly the poor and the malnourished. Scientists have been attempting to augment the protein content either by N-fertilization or by genetic manipulations. Significant progress has been made in understanding the factors affecting grain protein content and the complexity of inheritance of the trait. Though, seed protein content has been known to show a negative correlation with yield, the capacity to combine greater yield and high protein content has been reported in cereals like wheat and oats (Vasal 2002). Exploitation of the reservoir of genetic variability present in the landraces and germplasm for conventional breeding programs is an effective method for improving protein content of rice.

Rice seed storage proteins accumulate in two types of protein bodies-PB1 and PB II. The former is indigestible and negatively affects protein quality. Grains richer in prolamin fraction of proteins are not considered to be of high nutritional quality as the fraction has low levels of lysine, arginine and histidine.

#### 2.1. Achievements in high protein rice research

The trait for high grain protein content (GPC) has been transferred to high yielding background of many cereals such as wheat and oat (Vasal 2002) and subsequent marker validation has also been achieved. In case of rice, many QTLs along with the associated markers have been identified for ensuing transfer of GPC to high yielding background. But due to low heritability and significant influence of crop nutrient



management practices, improvement of rice varieties for this quantitative trait through simple breeding scheme was considered a real challenge. The ICAR-NRRI, Cuttack pioneered research and developed two high protein rice varieties in 2016.

A wide range of variability observed by screening of world rice germplasm at International Rice Research Institute indicated that GPC varied from about 5% to 18% with an average of 9.5% which indicated the possibility for improvement of rice GPC. Pedigree and long cycle recurrent selection were followed in earlier rice breeding programmes at IRRI, Philippines and negative correlation between yield and protein content was reduced to a significant level. However, the developed high protein lines were not accepted either due to deviation in grain type and cooking qualities from the adapted parent IR 8, or due to low stability of their protein yield. In a hybrid between IR 64 and O. nivara, the expression of the prolamin band was much intense than the parents. But the nutritional value of prolamin is inferior to that of glutelin for its low digestibility and negative influence on cooking quality as it increases the hardness of cooked rice. An inbred, high-protein, rice cultivar development project was initiated at the Louisiana State University Agricultural Center in 2005. The rice 'Frontière' containing 11% protein (Reg. No. CV-150, PI 674794) was the first cultivar produced by this project, which was released in 2015. It was developed from the cultivar Cypress through induced mutation by means of cellular selection. It had excellent milling quality (60.5% whole grain and 68.9% total milling yield), an average amylose content of 21.8%, and an intermediate gelatinization temperature (Wenefrida et al. 2017). Researchers from the University of Agricultural Sciences (UAS) developed a high protein rice strain, which has 12 to 13% protein in grain and a 20% increase in the amount of lysine, an essential amino acid (Satish Kumar 2016). A protein-enriched rice variety developed by Indira Gandhi Krishi Vishwavidyalaya (IGKV) with over 10% grain protein (which is 3% more than what is found in popular varieties) and 30 ppm zinc (Rewari 2016).

The ICAR-NRRI, Cuttack has evaluated about 3000 rice germplasm for grain protein content since 2004 and found wide diversity for the trait (5-15%). Two low yielding germplasm from Assam rice collection (ARC10075 and ARC10063) with high grain protein content (13-15%) in brown rice were identified (Table 1). The Institute has developed protein rich lines in high yielding backgrounds of popular varieties Naveen

Cultivars/ genotype	Crude Protein content (%) in brown rice	Cultivars/ genotype	Crude Protein content (%) in brown rice
PB-140	12.5-15.45	Bindli	11.93-13.8
CR Dhan-310	11.5-12.5	PB-84	12.2-13.8
ARC-10075	11.5-12.5	PLN-100 (CR Dhan311)	11.5-12.4
Kalinga-III	11.2-11.9	PB-177	11.8-13.59
PB-312	14.3-15.79	Naveen	7-7.5
Mamihungar	12.5-13.5	Swarna	6.9-7.5
PB-170	12.6-14.15		

#### Table 1. High protein rice varieties identified by ICAR-NRRI, Cuttack.



and Swarna, which suit irrigated and favourable rainfed system. Stability for yield and protein yield were tested for three years. They were used in bulk-pedigree and backcross breeding programme. The following two varieties were released in 2016 by NRRI, Cuttack with high protein content:

- High Protein rice CR DHAN 310 was released by CVRC in 2016. This is the first high protein (10.2%) rice variety at national level and has medium slender grains. This is an introgression line (CR 2829-PLN-37) in Naveen background. The average grain yield at national level was 4.5 t ha<sup>-1</sup>.
- Nutrient rich rice MUKUL (CR Dhan 311) (IET 24772: CR2829-PLN-100) was released in Naveen background by SVRC, Odisha in 2016. It has high protein (10.1%) and moderately high level of Zn (21 ppm). It is medium early (125 days) with long bold grain. The average yield at national level was 4.1 t ha<sup>-1</sup>.

Higher quantity of both  $\alpha$ -and  $\beta$ -glutelin in some of the high protein lines indicated enhancement of nutritional quality of this variety. These breeding lines did not show any significant changes in the intensity of prolamin band. Moreover, the prolamin/



Fig. 1. Rapid color test (xanthoproteic test) to distinguish between low and high protein grains

glutelin ratio in high protein lines either remained the same or was lower as compared to Swarna, indicating that the protein quality either remained the same or was improved by the breeding process.

A simple and rapid method to differentiate between the parent Naveen (7.5% protein) and its high protein version CR Dhan 310 was developed by ICAR-NRRI. It was found that the xanthoproteic test, the qualitative test used for confirming the presence of protein in a sample could easily distinguish between the two varieties on the basis of intensity of color produced (Fig. 1).

#### 2.2. Challenges and way forward

Scientific knowledge regarding the process of nitrogen uptake, assimilation and partitioning leading to the significant difference in high and low protein grains needs to be analyzed. The amino acid profiling also needs to be carried out to identify lysine/threonine rich varieties, because threonine forms a bigger part of the digestive tract mucosal proteins. Screening of more germplasms should also be an integral part of future program. The available germplasm is to be explored for nutrient dense rice with higher protein content, biological value and better amino acid profile. Further characterization of high protein rice cultivars/germplasm for resistant starch, bioavailability of Zn, amino acid profile, antioxidant value and phytic acid needs to be carried out. Biofortification using biotechnological approaches needs to be used as a



sustainable alternative to develop protein rich rice with higher amounts of lysine and threonine to meet the daily nutritional requirements of humans.

### **3. GLYCEMIC INDEX OF RICE**

The concept of glycemic index (GI) of foods developed by David Jenkins, Thomas Wolever and colleagues at the University of Toronto in 1981 ranks the quality of individual carbohydrate-rich foods on a scale of 1-100 by measuring how blood glucose levels rise after someone eats an amount of food that contains 50 grams of available carbohydrate. Foods are classified as low GI (GI, 55 or less), medium GI (GI, 56-69) and high GI (GI, 70 or more) types, when D-glucose is given a GI of 100. Foods with a low GI score provide steady fuel to support energy levels and overall health, while those with a high GI score are likely to provide an unhealthy quick rush of blood sugar followed by a sharp crash. Refined, processed starches/fruits have higher GI. Whole grains, high fiber foods, whole fruits, vegetables and legumes have lower GI.

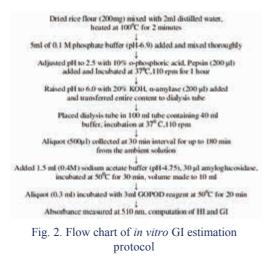
People living sedentary lifestyle and overeating foods, especially rice are likely to invite some health complications like type II diabetes and obesity. Presently, more than 62 million individuals are diagnosed with diabetes in India, which may rise to 79.4 million by 2030. This would involve huge financial burden on treatment of people. The GI value of rice varies from 48-92. A high dietary glycemic load (predominantly from rice) has been associated with increased risk of type 2 diabetes mainly in Chinese, Japanese and Indian populations. The development of low GI rice becomes a priority, as rice is eaten by a large number of people, many of whom are diabetics, mainly with type II diabetes.

#### 3.1. Achievements

Rice is nearly 90% carbohydrate on dry weight basis. The average GI value of rice (including the brown and milled grains) is 64 (Atkinson et al. 2008). Rice contains less than 3% RS (mainly of type 5) which escapes digestion almost entirely and therefore, its calories are unavailable for cells to use. RS positively influences the functioning of the digestive tract, microbial flora, blood cholesterol level, GI and helps to control diabetes (Fuentes-Zaragoza et al. 2010). The more the RS, the slower the digestion of rice and consequently the lower is the GI. Several investigators have reported that varieties with high-amylose content (AC) exhibit lower GI values than the low-amylose varieties (Denardin et al. 2007). The GI and RS contents have been established as important indicators of starch digestibility. Varieties with low GI and high RS content tend to lower the glycemic response (GR) due to slow release of glucose in small intestine, thus lowering the insulin response and controlling the rise in blood glucose (Englyst et al. 1992). In a study of US men and women, a moderate inverse association between diabetes risk and brown rice consumption was observed, although contradictory reports have also been received (Sun et al. 2010). Dietary intervention methods form the cornerstone of diabetes prevention/ management and are primarily aimed at maintaining a low and stable postprandial blood glucose level. There is evidence to suggest that low GI diets reduce the incidence of diabetes, hyperlipidaemia



and cardiovascular disease. Glycemic index values of milled rice of popular Indian varieties are higher (70-77) compared to those of brown rice (50-87) as per the 2008 international GI table.



At the ICAR-NRRI, more than 100 rice cultivars from different ecologies have been evaluated for GI and RS content of brown and milled rice. Further, the in vitro method for GI estimation of Goni et al. (1997) was improved (Fig. 2). Large variations in the values of GI (60-70), RS (0.35-2.57%) and AC (03.79-23.32 %) were observed. Among the genotypes studied, Mahsuri had lowest GI (60) and highest RS (2.57%). The highest value for GI (70) was found for Abhishek with relatively low RS (0.83%). O. brachyantha grains

had the lowest RS content (0.35%) with relatively high GI (69) (Fig. 3). Expression analysis of *gbssI* in developing grains of three rice genotypes differing widely in GI, RS and AC resulted in maximum expression in Mahsuri at middle stage showing a positive correlation between RS content and *gbssI* expression (Kumar et al. 2017).

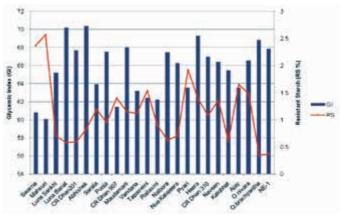


Fig. 3. Correlation between GI and RS values of different rice genotypes

#### 3.2. Challenges and way forward

Presently, very little information is available on GI values of processed, pigmented and scented rice. Similarly, comparative studies on GI of brown and milled rice of popular varieties are also rare. The role of degree of branching of amylopectin is also not understood. There is little information available on how addition of different



foods to rice affects the starch digestibility and GI value. An effective approach, to help check diabetes would be to screen all available germplasm for GI, AC, fiber and RS and popularize low GI rice among the rice eating population. Breeding for low GI rice can also be attempted by transferring the trait to popular high yielding varieties. As rice is often eaten with pulses, vegetables, curd, milk, cooking oils, and condiments etc., their effect on GI of rice also needs to be studied. Effect of processing, soaking and cooking methods on GI also needs attention.

## 4. ANTIOXIDANT VALUE OF RICE

Antioxidants, the substances found in foods and dietary supplements help protect cellular constituents like proteins, lipids and DNA against the damage caused by free-radicals including reactive oxygen species (ROS), which are routinely produced during aerobic energy metabolism in our body. Brown rice (BR) or dehusked rice, which is obtained when paddy (rough rice) is subjected to hulling is rich in bioactive components such as dietary fiber, functional lipids, amino acids, vitamins, phytosterols, phenolic compounds, gamma-aminobutyric acid (GABA), minerals and many antioxidant molecules. Flavonoids, the water soluble polyphenolic molecules containing 15 carbon atoms are a group of plant metabolites, which are thought to provide health benefits through cell signalling pathways and antioxidant effects and are divided into six major subtypes, which include chalcones, flavones, isoflavonoids, flavanones, anthoxanthins and anthocyanins. Ramarathnam et al. (1989a; 1989b) first identified the flavonoid isovitexin, alpha-tocopherol, and gamma-oryzanol in rice as having antioxidant activities comparable to that of butylated hydroxyanisole, (BHA), a common food preservative. To satisfy consumer's needs, the rice grains are usually milled into white rice, while the bran and husk are discarded. Most of the antioxidants are confined to the bran layer and endosperm and thus are largely absent from the milled rice. Pigmented rice is now gaining popularity because of its documented health benefits. In addition to its high protein, vitamin, and fiber content, it is a good source of a variety of phytochemicals including polyphenols, isoflavones, phytosterols, and anthocyanidins that have several beneficial functions in human health. The nutritional advantages offered by both brown and pigmented rice necessitate their inclusion in the daily diet to a greater extent. Hence, characterization of the colored and other rice for their antioxidant value needs to be a priority.

#### 4.1 Achievement

Pigmented rice have been documented to be rich in protein, vitamins, fiber, phytochemicals, such as polyphenols, isoflavones, phytosterols, and anthocyanidins which provide several health benefits. They have been found to be high in bioactive compounds like phenolics, tocols and sterol derivatives presenting antioxidant, antiinflammatory and other benefits. Polyphenols, such as phenolic acids, anthocyanins, and proanthocyanidins, have been reported as the major antioxidants in rice. Generally, white rice contains mainly phenolic acids; red rice is characterized by the presence of procyanidins, whereas black rice is characterized by the presence of anthocyanins.



The distribution of phenolic acids exhibits varietal differences; rice bran had the highest total phenolic content (TPC) among four different fractions of whole rice grain. Overall, ferulic, p-coumaric, isoferulic, syringic, vanillic, sinapic, caffeic, p-hydroxybenzoic, and protocatechuic acid are present in the whole rice grain, of which ferulic acid is the most abundant phenolic acid in the insoluble-bound fraction. The phenolic contents were positively correlated with the antioxidant capacity. In black rice, anthocyanins accumulate in the outer layers as free forms and cyanidin-3-O-glucoside and peonidin-3-O-glucoside have been identified in black rice bran as the main anthocyanin components. Pang et al. (2018) studied the total phenolic content (TPC), individual phenolic acid and antioxidant capacity of whole grain and bran fraction of 18 rice varieties with different bran color. The levels of TPC in bound fractions were found to be significantly higher than those in the free fractions either in the whole grains or bran. The study also concluded that there is wider diversity in the phenolics and antioxidant capacity in the whole grain and bran of rice.

Fourteen pigmented hill rice cultivars were studied for ascertaining the extent of their nutritional and genetic diversity. The total phenolic content was almost 50% higher in the pigmented rice as compared to non-pigmented rice. The range of DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging activity of 14 pigmented hill rice cultivars varied from 19.56% in Maichukik to 29.29% in Joradhan with an average value of 23.80%; no activity was detected in non-pigmented rice Vandana. Both the yellow and black varieties of the medicinal rice Njavara had higher antioxidant activity, higher bioactive compound content and higher anti-inflammatory activities than the staple rice varieties. Kaur et al. (2017) reported strong positive correlation between total phenolics and the antioxidant activity in non-pigmented rice varieties, whereas phytic acid content was negatively correlated with the antioxidant activity.

Research at the ICAR-NRRI, Cuttack showed that the total anthocyanin content (TAC), TPC and antioxidant activity (ABTS) differed significantly among the pigmented genotypes with highest values of these parameters in the purple grain (Mamihungar), whereas no significant difference between the colour groups (red and purple) was observed for total flavonoid content (TFC), gamma-oryzanols and phytic acid content indicating that value of these parameters depends on genotypes and not on kernel color. A high correlation of TAC with TPC and ABTS suggests that the major phytochemicals responsible for the tested antioxidant activities are phenolic acids and anthocyanins (Sanghamitra et al. 2017). Recently, a cross between Manipuri black rice and a white rice has resulted in grains with an almost black endosperm at NRRI. The variety may provide health benefit due to its high antioxidant content.

#### 4.2 Challenges and way forward

Although a large number of pigmented rice are grown in different pockets of India, there is little information on the types of such landraces, their area of cultivation, nutritional composition (especially with respect to antioxidant molecules) and the possible health benefits. Total antioxidant activity is significantly contributed by tocopherols, tocotrienols, and gamma-oryzanols (shown to reduce blood cholesterol level). Hence, different pigmented and white rice need to be evaluated for individual



antioxidant including gamma oryzanols. The genetic diversity found in the cultivars may be considered as a need to select the best performers amongst themselves with respect to the contents of nutritive and antioxidant principles as well as the antioxidant activity. Promising candidates thus selected can be utilized for developing improved lines in future breeding programmes.

## 5. MINERAL BIOAVAILABILITY

In Asia, rice serves as the major source of energy, protein, iron (Fe), zinc (Zn) and calcium (Ca) in the diet. Fe and Zn are essential trace elements in human nutrition and their deficiencies are major public health threats worldwide. Unfortunately, rice does not furnish minerals adequately, because it contains only small amounts of Fe and Zn, and the loss of minerals (particularly Fe) during milling is high. In addition, rice contains phytic acid (PA), the most important anti-nutritional factor impeding availability of divalent cations. Approximately 70% of total phosphorus in seeds coexists with PA and its content typically accounts for 1% or more of seed dry weight. As an anti-nutrient, high levels of PA can affect the bioavailability of essential minerals such as Zn, Fe and Ca, as it is a strong chelator of divalent cations. The anti-nutritional properties of PA can be further extended to human health, as it is considered to be the most important anti-nutritional factor contributing to the iron deficiency suffered by over 2 billion people worldwide. Phytic acid also has the potential to bind charged amino acid residues of proteins, with the concomitant reduction of protein availability. The undesirable properties of PA make the development and characterization of low phytate crops a high priority in agricultural research.

Nearly 80% Indians consume rice as a staple food making it the major source of carbohydrate and micronutrients such as Fe, Zn and vitamins. However, Fe and Zn content of rice grains is low, which is further reduced significantly during processing and by the presence of PA, which further limits their bioavailability. Micronutrient malnutrition, particularly, Zn deficiency affects 49% of the world population that accounts for 3-4 billion people, while over 2 billion people suffer from Fe deficiency worldwide. It is thus necessary to improve net content and bioavailability of both Fe and Zn from rice grain by identifying Fe, Zn rich and low PA rice cultivars.

#### 5.1. Achievements

Kiers (2000) reported that though the cereals and legumes are rich source of nutrients and antioxidants, they also contain indigestible constituents such as nonstarch polysaccharides, phenolic compounds, tannins and phytic acid. Brown rice is richer than milled rice in terms of protein, fat, vitamins and minerals but also has higher amounts of dietary fiber and phytate that may inhibit absorption of minerals. Phytic acid, considered as a significant inhibitor of minerals can form strong complexes with Fe and Zn in grains and limits the bioavailability of these microelements thereby reducing the nutritional value (Raboy 2001). Phytate is indigestible to humans or non-ruminants due to lack of appropriate digestive enzymes and is thus excreted as sanitary sewage into waterways resulting in eutrophication of water bodies (Swick



and Ivey 1992). In addition, phytate also inhibits the enzymes (like amylase, pepsin and trypsin) that digest food in the gut. Food fortification has been recommended as one of the preferred approaches for preventing and eradicating Fe and Zn deficiency. The bioavailability of minerals in rice can also be enhanced by selecting varieties with inherently high mineral content and cultivating them under good agricultural practices together with screening of lines with low PA and high mineral content. Considering the world-wide deficiencies of Fe and Zn, research work has been initiated to find the extent to which grain PA affects the bioavailability of Fe/Zn and to identify varieties that exhibit highest bioavailability of the two minerals. Out of the 70 rice varieties analyzed for PA content, six were evaluated for Fe and Zn bioavailability from brown and milled rice. An inverse relationship was found between PA content and Fe/Zn bioavailability in the brown rice of these varieties. Bindli, which had the lowest PA (0.82%), showed highest Zn bioavailability (21%), while PB267, which had the highest amount of PA (2.62%) showed low bioavailability of Zn (18%) and Fe (26%) (Kumar et al. 2017). The bioavailability of Zn increased after milling because most of the amount of this mineral is present in endosperm, while that of Fe decreased, most likely because the major amount of Fe is found in the bran layer (Fig. 4 and 5).

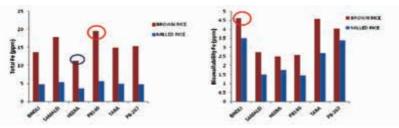


Fig. 4. Total Fe content and its bioavailability in brown and milled rice of six cultivars with contrasting PA content.

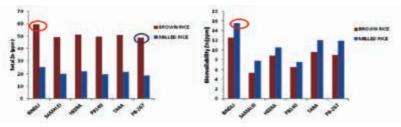


Fig. 5. Total Zn content and its bioavailability in brown and milled rice of six cultivars with contrasting PA content.

#### 5.2 Challenges and way forward

The information on PA value of brown and milled rice of most popular Indian varieties is not available. Similarly, information about other compounds present in rice grains which may affect minerals bioavailability along with PA is not available. Screening of rice genotypes, identification of those with low PA and high mineral



bioavailability and their popularization would help those suffering from micronutrient malnutrition. Research needs to be initiated for the assessment of impact of certain indigestible constituents such as non-starch polysaccharides, phenolic compounds and tannins on minerals bioavailability. The minimum safe content of PA in seed needs to be determined and studies on grain specific phosphorus transporter are required to help develop low phytate lines.

#### References

- Atkinson FS, Foster-Powell K and Brand-Miller JC (2008) International Tables of Glycemic Index and Glycemic Load Values. Diabetes Care 31(12):2281-2283.
- Denardin CC, Walter M, da Silva LP, Souto GD and Fagundes CAA (2007) Effect of amylose content of rice varieties on glycemic metabolism and biological responses in rats. Food Chemistry 105:1474-1479.
- Englyst HN, Kingman SM and Cummings JH (1992) Classification and measurement of nutritionally important starch fractions. European Journal of Clinical Nutrition 46:33-50.
- Fuentes-Zaragoza E, Riquelme-Navarrete MJ, Sanchez-Zapata E and Perez-Alvarez JA (2010) Resistant starch as functional ingredient: a review. Food Research International 43:931-942.
- Juliano BO, Cagampang GB, Cruz LJ and Santiago RG (1964) Some physicochemical properties of rice in Southeast Asia. Cereal Chemistry 41:275-286.
- Kaur M, Asthir B and Mahajan G (2017) Variation in Antioxidants, Bioactive Compounds and Antioxidant Capacity in Germinated and Ungerminated Grains of Ten Rice Cultivars. Rice Science 24(6):349-359.
- Kumar A, Kar SS, Lal MK, Nayak L, Ngangkham N, Samantaray S and Sharma SG (2017) Bioavailability of Iron and Zinc as affected by Phytic acid content in rice grain. Journal of Food Biochemistry 41(6):e12413.
- Kumar A, Sahoo U, Baisakha B, Okpani OA, Ngangkham U, Parameswaran C, Basak N, Kumar G and Sharma SG (2018) Resistant starch could be decisive in determining the glycemic index of rice cultivars. Journal of Cereal Science 79:348-353.
- Pang Y, Ahmed S, Xu Y, Beta T, Zhu Z, Shao Y and Bao J (2018) Bound phenolic compounds and antioxidant properties of whole grain and bran of white, red and black rice. Food Chemistry 240:212-221.
- Raboy V (2001) Seeds for a better future: 'low phytate' grains help to overcome malnutrition and reduce pollution. Trends in Plant Science 6:458-462.
- Ramarathnam N, Osawa T, Namiki M and Kawakishi S (1989a) Chemical studies on novel rice hull antioxidants. 2. Identification of isovitexin, a C-glycosyl flavonoid. Journal of Agricultural and Food Chemistry 37:316-319.
- Ramarathnam N, Osawa T, Namiki M and Kawakishi S (1989b) Studies on changes in fatty acid composition and content of endogenous antioxidants during c irradiation of rice seeds. Journal of the American Oil Chemists' Society 66:105-108.
- Rewari G (2016) New protein-rich rice developed by IGKV will help millions of undernourished people. Rice Outlook. October 25, 2016 (http://riceoutlook.com/new-protein-rich-rice-developed-by-igkv-will-help-millions-of-undernourished-people/).
- Sanghamitra P, Bagchi TB, Sah RP, Sharma SG, Sarkar S and Basak N (2017) Characterization of red and purple-pericarp rice (*Oryza sativa* L.) based on physico-chemical and antioxidative properties of grains. Oryza 54(1):57-64.

447



- Satish Kumar BS (2016) A more muscular rice variety takes on wheat. The Hindu, December 09, 2016 (http://www.thehindu.com/sci-tech/agriculture/A-more-muscular-rice-variety-takes-on-wheat/article16780286.ece).
- Sun Q, Spiegelman D, van Dam RM, Holmes MD, Malik VS, Willett WC and Hu FB (2010) White rice, brown rice, and risk of type 2 diabetes in US men and women. Archives of Internal Medicine 170:961-969.
- Swick RA and Ivey FJ (1992) The value of improving phosphorus retention. Feed Management 43:8-17.
- Vasal SK (2002) The role of high lysine cereals in animal and human nutrition in Asia. In: Protein Sources for the Animal Feed Industry, Food and Agriculture Organization, Rome, pp 167-183.
- Wenefrida I, Utomo HS and Linscombe, SD (2017) Development and Registration of 'Frontière', a High-Protein Rice Cultivar. Journal of Plant Growth Regulation 11:240-244.\*



## Improvement of Photosynthetic Efficiency of Rice: Towards Sustainable Food Security under Changing Climate

#### MJ Baig, P Swain, K Chakraborty, A Kumar, KA Molla and G Kumar

#### **SUMMARY**

According to various estimates, we will have to produce 40% more rice by 2025 to satisfy the growing demand without affecting the resource base adversely. This increased demand will have to be met from less land, with less water, less labor and fewer chemicals. Most of our conventional crops, including rice and wheat, assimilate atmospheric CO<sub>2</sub> by the C<sub>3</sub> pathway of photosynthesis, which takes place in the mesophyll cells of leaves. Photosynthetically, these plants are underachievers because on the one hand, they assimilate atmospheric CO<sub>2</sub> into sugars but on the other hand, part of the potential for sugar production is lost by respiration in daylight, releasing CO<sub>2</sub> into the atmosphere, a wasteful process termed photorespiration. C<sub>4</sub> plants exhibit many desirable agronomic traits: high rate of photosynthesis, fast growth and high efficiency in water and mineral use. In this chapter we will discuss various aspects of improvement of photosynthetic efficiency and creation of a C<sub>4</sub> rice plant which has the potential to generate substantially higher farm yields and make an important contribution to global poverty alleviation efforts.

#### **1. INTRODUCTION**

Rice is an important food crop which feeds for more than half of the global population. It accounts for 35-75% of the calories consumed by more than 3 billion Asians as more than 90% of the world's rice is grown and consumed in Asia which is home to 60% of the human population. The crop is planted to nearly 154 million hectares annually which makes up 11% of the total cultivated land on the earth. About 75% of India's poor people with low purchasing power live in rural areas and nearly 60% of the cultivated area is under rainfed farming. According to various estimates, we will have to produce 40% more rice by 2025 to satisfy the growing demand without affecting the resource base adversely. This increased demand will have to be met from less land, with less water, less labor and fewer chemicals. If we are not able to produce more rice from the existing land resources, the hungry farmers will destroy forests and move into more fragile lands such as hillsides and wetlands with disastrous consequences for biodiversity and watersheds. To meet the challenge of producing more rice from suitable lands, we need the rice varieties with higher yield potential and greater yield stability. Most of our conventional crops, including rice and wheat, assimilate atmospheric CO<sub>2</sub> by the C<sub>2</sub> pathway of photosynthesis, which takes place in the mesophyll cells of leaves. Photosynthetically, these plants are



underachievers because on the one hand, they assimilate atmospheric CO<sub>2</sub> into sugars but on the other hand, part of the potential for sugar production is lost by respiration in daylight, releasing CO<sub>2</sub> into the atmosphere, a wasteful process termed photorespiration. This is due to the dual function of the key photosynthetic enzyme, Ribulose 1, 5-bisphosphate carboxylase/oxygenase (Rubisco). High CO<sub>2</sub> favors the carboxylase reaction and thus net photosynthesis, whereas high O<sub>2</sub> promotes the oxygenase reaction leading to photorespiration. Photorespiration reduces net carbon gain and productivity of C<sub>3</sub> plants by as much as 40%. This renders C<sub>3</sub> plants less competitive in certain environments. In contrast, with some modifications in leaf anatomy, some tropical species (e.g., maize and sugarcane) have evolved a biochemical "CO<sub>2</sub> pump," the C<sub>4</sub> pathway of photosynthesis to concentrate atmospheric CO<sub>2</sub> in the leaf and thus overcome photorespiration. Therefore, C<sub>4</sub> plants exhibit many desirable agronomic traits: high rate of photosynthesis, fast growth and high efficiency in water and mineral use.

The single cell  $C_{A}$  photosynthetic system has given us the clue that it may be experimentally feasible to genetically engineer all  $C_4$  genes in a single cell of  $C_3$  plants, say rice to enhance its photosynthetic activity and productivity. However, at the CO, compensation point, net CO, assimilation equals CO, release through photorespiration and mitochondrial respiration in light. In high CO<sub>2</sub> and /or low O<sub>2</sub> the oxygenase activity of Rubisco is virtually absent, the flux through the photorespiratory carbon cycle negligible and the CO, compensation point close to zero. Rice being a C, plant, the first product of atmospheric CO<sub>2</sub> fixation is the 3-carbon compound 3phosphoglycerate (3-PGA), which is produced during the Calvin cycle by Rubisco (the only enzyme capable of net carbon assimilation) in the chloroplast stroma. However, competition of O, with CO, at the active site of Rubisco results in a loss of up to 50% of the carbon fixed in a process known as photorespiration. Oxygenation of Ribulose-1,5-biphosphate (RubP) severely diminishes the efficiency of CO<sub>2</sub> assimilation in rice under ambient air and results in the formation of 3-PGA as well as 2-phosphoglycolate (2-PGA). The latter is metabolized in the compartments of the leaf cell, the chloroplast, the peroxisomes and the mitochondria, involving numerous enzymatic reactions and transport processes. The overall photorespiratory cycle is also linked to amino acid metabolism in that glycine, serine, glutamate and glutamine are metabolized at high rates. Both CO<sub>2</sub> and ammonia are released at equal rates in the reaction catalyzed by the mitochondrial glycine decarboxylase complex. By introducing the Escherichia coli glycolate catabolic pathway into rice, the loss of fixed carbon and nitrogen due to photorespiration can be reduced to a great extent. Using stepwise transformation with five chloroplast-targeted bacterial genes encoding glycolate dehydrogenase, glyoxylate carboligase and tartronic semialdehyde reductase, the plants may be generated in which chloroplastic glycolate is converted directly to glycerate. This would reduce, but not eliminate, flux of photorespiratory metabolites through peroxisomes and mitochondria. Such plants thus, may grow faster, produce more shoot and root biomass, and may contain more soluble sugars, reflecting reduced photorespiration and enhanced photosynthesis that correlate with an increased chloroplastic CO2 concentration in the vicinity of Rubisco. These effects are evident after over-expression of the three subunits of glycolate dehydrogenase but were enhanced by introducing the complete bacterial glycolate catabolic pathway. Diverting



chloroplastic glycolate from photorespiration may improve the productivity of rice with  $C_3$  photosynthesis.

Atmospheric CO<sub>2</sub> concentrations has increased significantly in the past two centuries, from 270 µmol mol<sup>-1</sup> in 1750 to current concentrations which exceed 400 µmol mol<sup>-1</sup>. The primary effects of elevated CO<sub>2</sub> levels in most crop plants, particularly C3 plants, include increased biomass accumulation, although initial stimulation of net photosynthesis rate is only temporal and plants fail to sustain the maximal stimulation, a phenomenon known as photosynthesis acclimation. Increase in CO<sub>2</sub> has double effect on C<sub>3</sub> (rice) plants such as an increase in leaf photosynthesis and a decrease in stomatal conductance to water vapor. Elevated CO<sub>2</sub> levels increase net leaf photosynthetic rate primarily by competitive inhibition of the oxygenase activity of Rubisco and therefore photorespiration; and by acceleration of carboxylation because the CO<sub>2</sub> binding site is not saturated at the current CO<sub>2</sub> levels. Rubisco catalyzes the competitive reactions of RuBP carboxylation and RuBP oxygenation. It has long been recognized that genetic modification of Rubisco to enhance its specificity for CO<sub>2</sub> relative to O<sub>2</sub> would decrease photorespiration and potentially increase C<sub>3</sub> photosynthesis and correspondingly crop productivity. Although Rubisco has been the primary focus of research to improve photosynthetic efficiency, it has been clearly demonstrated that metabolic control of CO<sub>2</sub> fixation rate is shared among different enzymes in the pathway.

The enhancement of photosynthetic efficiency has emerged to provide a vital opportunity to address the challenge of sustainable yield increases needed to meet future food demand. Attaining higher photosynthesis rates for the same or decreased use of water and nitrogen resources could be the crucial point to transform the agriculture of the twenty-first century.

The CO<sub>2</sub> concentrating mechanism, together with modifications of leaf anatomy, enables C<sub>4</sub> plants to achieve high photosynthetic capacity and high water and nitrogen use efficiencies and ultimately high yield. As a consequence, the transfer of C<sub>4</sub> traits to C<sub>3</sub> plants is one strategy being adopted for improving the photosynthetic performance of C<sub>3</sub> plants. Improving the photosynthetic efficiency and creation of a C<sub>4</sub> rice plant has the potential to generate substantially higher farm yields and make an important contribution to global poverty alleviation efforts.

### 2.PHOTOSYNTHESIS AND ITS CLASSIFICATION

Photosynthesis is the process by which plants, some bacteria, and some protistans transform light energy into chemical energy.

The overall reaction of this process is:  $6H_2O + 6CO_2 \longrightarrow C_6H_{12}O_6 + 6O_2$ 

#### 2.1. $C_3$ and $C_4$ photosynthesis

The difference occurs in the second part of photosynthesis, the Calvin-Benson cycle, which "fixes"  $CO_2$  into carbohydrates. As  $CO_2$  is used up by the normal Calvin-Benson cycle, the balance of  $CO_2$ :  $O_2$  inside the leaf alters in favor of  $O_2$ , and Rubsico starts to grab it instead. This both slows down photosynthesis and reduces its carbon fixation overall.



The C<sub>4</sub> plants have introduced an extra bit into the Calvin-Benson cycle, an extra early reaction that fixes CO<sub>2</sub> not into 3-carbon sugars, but 4-carbon compound called oxaloacetate by plunking CO<sub>2</sub> onto a different receptor molecule (phosphoenolpyruvate, or PEP) by way of the enzyme PEP carboxylase, which has two advantages over Rubisco: it has no affinity for O<sub>2</sub> at all, and it finds and fixes CO<sub>2</sub> even at very low CO<sub>2</sub> levels. Oxaloacetate has an advantage over 3PG, in low CO<sub>2</sub> circumstances some of it degrades to form CO<sub>2</sub> again in the mesophyll, the cells which carry CO<sub>2</sub> to Rubisco. As a result, the C<sub>4</sub> plants can close their stomata to retain moisture under hot, dry conditions, but still keep photosynthesis ticking over at good efficiency.

#### 2.2. Crassulacean acid metabolism (CAM) plants

Crassulacean acid metabolism plants (from "crassulacean acid metabolism", because this mechanism was first described in members of plant family Crassulacean) are different kind of  $C_4$  plants. In the  $C_4$  plants described above, the fixation of  $CO_2$  into 4-carbon sugars and the further fixation of  $CO_2$  into 3-carbon sugars happen in different cells, separated in space but at the same time in CAM plants, the two different kinds of  $CO_2$ -fixation reactions happen in the same cells, which separated in time. In CAM plants the fixation of  $CO_2$  into oxaloacetate happens at night, when it is cooler and the stomata can open to ensure a plentiful supply of  $CO_2$ , and then the oxaloacetate is stored as malic acid. During the day, the stomata close to minimize moisture loss, and the stored malic acid is reclaimed and turned back into  $CO_2$  to power the normal Calvin cycle.

#### 2.3. Single cell $C_4$ photosynthesis

It has been thought that a specialized leaf anatomy composed of two distinctive photosynthetic cell types (Kranz anatomy) is required for C<sub>4</sub> photosynthesis, which can function within a single photosynthetic cell in terrestrial plants. Borszczowia aralocaspica (Chenopodiaceae) has the photosynthetic features of  $C_{4}$  plants yet lacks Kranz anatomy. This species accomplishes C4 photosynthesis through spatial compartmentation of photosynthetic enzymes, and by separation of two types of chloroplasts and other organelles in distinct positions within the chlorenchyma cell cytoplasm. The most dramatic variants of C<sub>4</sub> terrestrial plants were discovered recently in two species, Bienertia cycloptera and Borszczowia aralocaspica, each has novel compartmentation to accomplish C<sub>4</sub> photosynthesis within a single chlorenchyma cell. The C<sub>4</sub> photosynthesis in terrestrial plants was thought to require Kranz anatomy because the cell wall between mesophyll and bundle sheath cells restricts leakage of CO<sub>2</sub>. Recent work with the central Asian chenopods Borszczowia aralocaspica and Bienertia cycloptera show that C<sub>4</sub> photosynthesis functions efficiently in individual cells containing both the  $C_4$  and  $C_3$  cycles. These discoveries provide new inspiration for efforts to convert C3 crops into C4 plants because the anatomical changes required for C<sub>4</sub> photosynthesis might be less stringent than previously thought (Fig. 1 depicts the different types of photosynthesis and process).



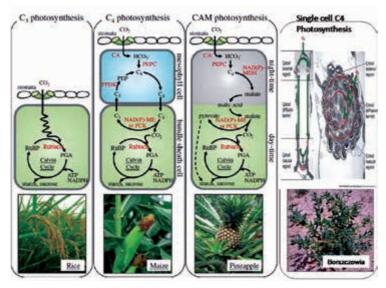


Fig.1. Different type of photosynthesis (plants and process)

The present chapter focuses on the advantages offered by  $C_4$  plants over  $C_3$  plants under adverse climatic conditions. Further we have tried to explore the different mechanism of increasing photosynthetic efficiency and conversion of  $C_3$  rice into  $C_4$  rice to ensure enhanced rice productivity under the changing climatic scenario.

### 3. ACHIEVEMENTS IN IMPROVEMENT OF PHOTOSYNTHETIC EFFICIENCY OF RICE AND EFFORTS TO CONVERT C<sub>3</sub> RICE INTO C<sub>4</sub> RICE

#### 3.1. Improving light utilization efficiency by altering the plant architecture

Light use efficiency (LUE) plays a key role in determining the photosynthetic efficiency of crop plants and thus has considerable impact on biomass production and yield. Important components of LUE are canopy structure, nitrogen utilization, photosynthetic capacity and  $CO_2$  diffusion rate. Modification of plant types or architecture is thought to be an important strategy to enhance the photosynthetic efficiency and potential yield of crops. Photosynthetic efficiency is found to vary linearly with leaf nitrogen content, independent of the canopy position. Rice leaves at early tillering stage under well fertilized N-treatments had higher photosynthetic rates due to higher leaf N content leading to greater amounts of rate-limiting photosynthetic proteins, which gave them an early head start and boost in productivity and leaf area index (LAI), bringing increases in canopy light interception (Xue et al. 2016).

Plant architecture-the three-dimensional organization of the above ground plant parts encompasses branching (tillering) pattern, plant height, arrangement of leaves

453



and the structure of reproductive organs. Plant architecture is of major agronomic importance as it determines the adaptability of a plant to cultivation, its harvest index and potential grain yield. Rice plant architecture is mainly determined by tiller pattern, plant height, leaf shape, arrangement and panicle architecture (Yang and Hwa 2008). The canopy architectures of three typical hybrid rice cultivars were measured in field condition using 3-D modelling methodology at four development stages from the panicle initiation to the filling stage, where structural parameters of the rice canopies were calculated and their light capture and potential carbon gain were simulated based on a 3-D light model. The study showed that erect plant type with steeper leaf angles allows light to penetrate more deeply with relatively uniform light distribution in the canopy at higher sun elevation angles. The LUE at the higher leaf area index could be enhanced by reducing mutual-shading (Zheng et al. 2008).

Ideotype breeding of rice, also known as modification of plant architecture, as per Yuan's (1997) model for rice, includes important morphological traits viz. medium plant height for higher harvest index; moderately compact growth habit and moderate tillering capacity for optimum panicles; top three functional leaves that are more erect, thicker, longer and slight rolled for more efficient use of light energy and photosynthesis; and heavy, droopy heavy panicle for more dry matter accumulation (Yuan 1997). Improvement in the tiller angle makes plants more efficient at trapping light for photosynthesis, which also allows plants to avoid some diseases by decreasing humidity around plant canopy. The identification of tiller angle control (TAC) gene QTL, which controls tiller angle, indicates the possibility of creating new rice plant type with moderate tiller angle by altering the expression level of *TAC* via transgenic approach.

#### 3.2. Enhancing LUE by improving the leaf characters

Plant architecture, especially the arrangement of leaves and tillers in rice has considerable effect on governing the LUE and photosynthetic efficiency. In a field study with IR72, resulted linear relationship between leaf N concentration (on dryweight basis) and SPAD chlorophyll meter reading at mid tillering, panicle initiation and flowering stages on the uppermost fully expanded leaves of both N-deficient and N-sufficient plants. In rice, shading at grain filling stage increased the flag leaf chlorophyll content and maximum efficiency of PSII photochemistry under darkadaption ( $F_{\gamma}/F_m$ ), but decreased the net photosynthetic rate, electron transport rate (ETR), saturation irradiance ( $PAR_{sal}$ ) and maximum electron transport rate ( $J_{max}$ ), which resulted in sharp drop in grain yield mainly due to reduced spikelet filling and grain weight (Wang et al. 2015).

#### 3.3. Introduction of $C_4$ enzymes in rice

Rice is a model  $C_3$  plant which operates Calvin cycle for fixation of atmospheric  $CO_2$  into carbohydrate. The  $C_4$  cycle operating plants are suitable for the changing climatic scenario to address the global food security issue. Hence, scientists took initiative to transform rice into  $C_4$  plant, but the efforts made during last two decades to introduce the  $C_4$  mechanism into rice to increase photosynthetic efficiency and enhance yield did not meet with much success.

454



Enzymes that play crucial role in  $C_4$  pathway have been cloned from  $C_4$  species and over-expressed in transgenic rice plants.

**3.3.1. Phosphoenolpyruvate carboxylase (PEPC):** The key carbon fixing enzyme of  $C_4$ pathway is phosphoenolpyruvate carboxylase (PEPC), which catalyses the carboxylation of PEP to form the four-carbon molecule oxaloacetate (Sage et al. 2004). The C<sub>4</sub> specific PEPC genes from maize and other C<sub>4</sub> plants have been expressed in rice. Over-expression of maize PEPC gene in transgenic rice plants has been found to reduce O<sub>2</sub> inhibition of photosynthesis and enhance photosynthetic rates compared to untransformed plants. In another study, Jiao et al (2005) showed that maize PEPC expression in rice increased photosynthetic capacity about 50% under high CO<sub>2</sub> supply along with enhanced tolerance to photo-inhibition. The PEPC transgenic rice plants also exhibited significant increase in carbonic anhydrase (CA) activity. Similarly, when Indica rice was transformed with maize PEPC, the transgenic plants exhibited significantly elevated photosynthesis rate, stomatal conductance and internal CO<sub>2</sub>concentration, while no yield enhancement was observed. Chen et al. (2014) demonstrated that PEPC enzyme activity and photosynthesis in transgenic rice can be promoted by exogenous nitric oxide (NO) treatment. In a similar fashion, photosynthesis of transgenic rice plants expressing maize PEPC gene was found to be less sensitive to O<sub>2</sub> inhibition (Agarie et al. 2002). Their results suggested that the O<sub>2</sub>-insensitive photosynthesis in the PEPC transformants was caused by a Pi limitation of photosynthesis. The sugarcane PEPC gene in rice has been demonstrated to increase the rate of photosynthesis under high temperature, (Lian et al. 2014). Rice plant has its endogenous C<sub>3</sub> PEPC which plays an aplerotic role by replenishing the TCA cycle intermediates that are withdrawn for nitrogen assimilation and for different biosynthetic pathways (Izui et al. 2004). The C<sub>4</sub> PEPC has evolved from an ancestral non-photosynthetic C<sub>3</sub> PEPC and during the course of evolution; C<sub>4</sub> PEPC has increased its kinetic efficiency as well as reduced its sensitivity to the feedback inhibitors malate and aspartate. The reduction in inhibitor affinity and increased kinetic efficiency of  $C_4$  PEPC are due to the substitution  $Arg_{(C3)}$ 884>Gly<sub>(C4)</sub> and Ala (C3) 774>Ser<sub>(C4)</sub> respectively.

**3.3.2. Malate dehydrogenase (MDH):** Malate dehydrogenase catalyzes the reduction of oxalate to malate in the second step of  $C_4$  cycle. Overexpression of sorghum NADP-malate dehydrogenase enzyme in rice plant did not affect the photosynthetic  $CO_2$  assimilation rate.

**3.3.3. Pyruvate orthophosphate di-kinase (PPDK):** The regeneration of PEP, the primary CO<sub>2</sub> acceptor, from pyruvate is mediated by PPDK enzyme in the mesophyll cell. Several studies described the development of transgenic rice expressing C<sub>4</sub> specific PPDK gene. PPDK activity in transgenic rice plant was greatly increased when intact maize PPDK gene with promoter, intron and terminator was introduced, whereas introduction of only chimeric cDNA construct resulted in slight increase in enzyme activity. This indicates an important role for endogenous promoter, intron and terminator for high level of PPDK expression. In another study, introduction of maize *PPDK* gene in the rice IR 64 was shown to increase total nitrogen content of flag leaf by 42.1%, CO<sub>2</sub>assimilation rate as well as other yield forming factors like dry weight



and harvest index than the control plant (Zhang et al. 2010). No significant change in carbon assimilation in transgenic upland rice was observed when  $C_4$  PPDK gene from *Echinochloa* was expressed (Wang and Li 2008). The rice plant has its own PPDK gene, but very low level of expression makes it difficult to detect PPDK activity, which might be due to the absence of an operative regulatory mechanism like maize  $C_4$  PPDK. Except the weaker promoter strength for larger transcript, Rice  $C_4$ -like PPDK is very similar to the maize PPDK gene. It is evident from the previous studies that the overexpression of  $C_4$ -PPDK is not able to significantly impact the carbon metabolism of transgenic rice leaves. This might be due to the freely reversible nature of PPDK mediated reaction, which depends on other factors like concentration of substrates, activators and inactivators (Miyao et al. 2003). However, Gu et al. (2013) showed that maize PPDK expression in rice causes increase in leaf photosynthetic rate, higher yield and enhanced drought tolerance.

3.3.4. NADP-dependent malic enzyme (NADP-ME): The NADP-ME catalyzes the decarboxylation reaction, one of the key steps in  $C_4$  cycle, which increases CO<sub>2</sub> concentration in the vicinity of Rubisco in bundle sheath cells. Transgenic rice plants expressing NADP-ME from maize (Tsuchida et al. 2001) and sorghum (Chi et al. 2004) have been reported. A rice- C3 specific isoform has also been over-expressed in rice (Tsuchida et al. 2001). Although activity of NADP-ME has been found to be increased significantly in transgenic rice, the transgene failed to increase photosynthetic efficiency of transgenic rice (Tsuchida et al. 2001, Chi et al. 2004). Transgenic rice expressing rice C<sub>3</sub> specific isoform of NADP-ME did not exhibit any detectable difference in plant growth (Tsuchida et al. 2001), increased levels of maize NADP-ME enzyme in transgenic rice plants led to stunting and leaf photobleaching (Tsuchida et al. 2001). The increased NADPH/NADP ratio and suppressed photorespiration has been proposed as probable reason behind the enhanced susceptibility to photoinhibition (Tsuchida et al. 2001). However, no change was observed in the phenotype under normal growth condition of rice plant expressing sorghum NADP-ME (Chi et al. 2004). In subsequent study the basis of increased photo-inhibition was done and speculation were made that accumulation of C<sub>4</sub> specific NADP-ME led to NADP deficiency and photosystem I over-reduction, which in turn accumulates reactive oxygen species (ROS) in the transgenic rice plants and makes the plant more sensitive to photo-oxidation.

**3.3.5. Phosphoenolpyruvate carboxykinase (PCK):** Phosphoenolpyruvate carboxykinase is an enzyme which also catalyzes the decarboxylation steps in  $C_4$  cycle like NADP-ME but in a different group of plants known as PCK type. Transgenic rice plants expressing the  $C_4$ -PCK gene from *Urochloa panicoides* have been demonstrated to be able to partially change the carbon flow in mesophyll cells into a  $C_4$ -like pathway. In another study, Huang et al. (2008) demonstrated that expression of maize PCK enzyme resulted in enhanced growth and yield of transgenic rice plant.

#### 3.4. Pyramiding of transgenes

Although overproduction of single  $C_4$  enzymes in rice could alter the carbon metabolism, but no remarkable improvement in photosynthesis and plant yield has

456



been reported till now. Pyramiding of  $C_4$  genes is another viable option to positively impact rice photosynthesis. Several studies have been conducted to simultaneously express more than one  $C_4$  genes in rice. Enhanced stomatal conductance and higher internal CO<sub>2</sub> concentration were found to be associated with increased photosynthetic efficiency (up to 35%) in transgenic rice plants co-expressing the maize PEPC and PPDK genes. When PCK and PEPC genes were simultaneously overproduced, low chlorophyll concentration and swollen thylakoid phenotype were observed in transgenic rice. The result of the study suggested a little contributory effect of elevated PEPC activity in combination with PCK activity on  $C_4$ -like carbon flow. Interestingly, ATP treatment of transgenic rice co-expressing *PEPC* and *PPDK* genes increased net photosynthetic rate by 17 and 12% under high irradiance and high temperature, respectively.

Enhanced drought tolerance, higher photosynthetic rate and higher yield were observed in transgenic rice pyramided with *PPDK* and *PEPC* genes (Gu et al. 2013). The co-over-expression of four  $C_4$  enzymes, viz., PEPC, PPDK, MDH and ME has been studied. They developed transgenic rice plants with different combination of  $C_4$  enzymes and as well as with all four enzymes. Results of their study showed that only the transgenic plants with all 4 enzymes exhibited some improvements on photosynthesis, while other combinations of 2 or 3 enzymes could not show any improvements. However, transgenic rice plants overproducing the four enzymes exhibited slight stunting phenotype. They found that combinatorial expression of ME with PEPC is the responsible factor for height reduction. A recent study showed that pyramiding of CA, PEPC and PPDK genes in rice resulted in increased photosynthetic efficiency and grain yield (Sen et al. 2017). The three genes in transgenic plants also have been demonstrated to contribute towards increased root biomass, wider leaves and stronger stalks.

#### 3.5 Minimizing photorespiration in C<sub>3</sub> plants like rice

The over production of PPDK, MDH or ME did not affect the rate of photosynthetic CO, assimilation, while in the case of PEPC, it was slightly reduced. The restoration of CO<sub>2</sub> assimilation was more at higher concentration which is an indication that overproduction of the four enzymes in combination did not act to concentrate CO<sub>2</sub> inside the chloroplast. Transgenic rice with different levels of the introduced enzyme were compared and it was concluded that overproduction of a single  $C_{4}$  enzyme did not improve photosynthesis of rice. Even, overproduction of the maize PEPC slightly inhibited photosynthesis through stimulation of respiration in light and reduction of the Rubisco activity. Expression of ictB gene of Arabidopsis and Nicotiana tabacum in Cyanobacterium documented that photosynthetic rate was significantly faster than the wild types. E. coli glycolate catabolic pathway was introduced into chloroplast of Arabidopsis to reduce photorespiration. The plants had more shoot and root biomass with more soluble sugar and enhanced photosynthetic rate. By applying same principle, it was concluded that by relocating the photorespiratory CO<sub>2</sub> (released from mitochondria) into chloroplast, reduced energy cost by avoiding ammonia release. It was shown to be the main factor that contributed to an improved photosynthetic efficiency.

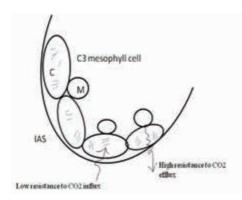


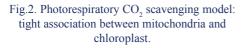
Expression of photorespiratory bypass genes in *Camelina* resulted in reduced photorespiration and increased photosynthesis in both partial and full bypass expressing lines. Expression of partial bypass increased seed yield by 50-57 %, while expression of full bypass increased seed yield by 57-73 %, without any loss in seed quality. The transgenic plants also showed increased vegetative biomass and faster development compared with wild type.

At National Rice Research Institute, the research work is being done on two aspects, namely, introduction of  $C_4$  genes in rice and reducing photorespiratory rate. For minimizing photorespiratory effect, the possible strategy is the introduction of bacterial (E. coli) glycolate catabolic pathway into rice chloroplasts to reduce the loss of fixed carbon and nitrogen and maintain photorespiration in plant. Many bacteria like E. coli can use glycolate as a sole carbon source. Five chloroplast-targeted bacterial genes encoding GDH, GCL and TSR, have been amplified by PCR from E. coli gDNA using suitable oligonucleotides and cloned in pGEMT vector. All sequences are available from the E. coli K12 genome sequence (gi49175990). Rice Rubisco smaller subunit (rbcs) transit peptide (~300 bp) nucleotide sequence has been also amplified and cloned in pGEMT vector. The transit peptide sequence is used for tagging into the GDH, GCL and TSR in order to facilitate transferring integrated genes product from nuclear genome to chloroplast genome. This will generate plants in which chloroplastic glycolate would be converted directly to glycerate. This would reduce, though may not eliminate, flux of photorespiratory metabolites through peroxisomes and mitochondria while increasing the rate of carbon fixation.

#### 3.6. Photorespiratory CO<sub>2</sub> scavenging mechanism

Though the  $CO_2$  released during the decarboxylation step of photorespiration in mitochondria is not completely lost and can be re-fixed while passing through the chloroplast by some plants, (photorespiratory  $CO_2$  scavenging mechanism), there is





barrier by chloroplast that can trap photorespiratory  $CO_2$ . This effect can be enhanced by a tight association between mitochondria and chloroplast (Busch et al. 2013) (Fig.2).

# 3.7. Introducing cyanobacterial CO<sub>2</sub> concentrating mechanisms (CCM) into chloroplasts

To introduce a cyanobacterial CCM in to the chloroplasts of land plant is another strategy to reduce oxygenation process of Rubisco. Cyanobacterial  $CO_2$  concentrating inside the proteinaceous micro-compartment called the carboxysome by which it suppresses the



oxygenating reaction of Rubisco. An outer shell called â-carboxysome (composed by several different proteins) encloses Rubisco and CA and maintains high  $CO_2$  concentration inside the micro-compartment and increases the catalytic efficiency of the carboxylation reaction of the enzyme. A potential approach is the engineering of a CCM into chloroplast of higher plant to express a functional cyanobacterial form of Rubisco together with proteins involved in the enzyme's assembly. However, the engineered plants were able to survive only at high  $CO_2$  concentration. The transformed plant expressed a rich amount of the foreign transporter but displayed the same  $CO_2$  assimilation rates as the wild type plant, indicating that the transporter had little or no *in vivo* activity.

#### 3.8. Photorespiratory bypass pathway

Despite some disadvantages, photorespiration has important role in plant because it recovers 75% of the carbon from phosphoglycolate as well as efficiently removes potent inhibitors of photosynthesis. Moreover, photorespiration dissipates excess photo-chemical energy under high light intensities, thus protecting the chloroplast from over-reduction. Instead of trying to reduce the photorespiration, the promising idea is to engineer photorespiratory bypass mechanism by introducing the *Escherichia coli* glycolate catabolic pathway to convert glycolate to glycerate directly in the chloroplast without ammonia release. This pathway would metabolize phosphoglycolate produced by RuBP oxygenation but minimize carbon, nitrogen and energy losses and avoid the accumulation of photorespiratory intermediates. Many studies suggested that this pathway requires less energy and shifts  $CO_2$  release from mitochondria to chloroplast. Improved  $CO_2$  fixation would not only increase the productivity of crop but also simultaneously decrease consumption of water, fertilizer and land (Fig. 3 & 4).

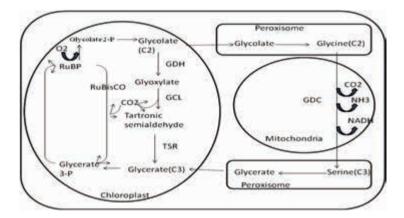


Fig. 3. Photorespiratory bypass pathway: -GDH (Glycolate gehydrogenase), GCL(Glyoxylate carboxyligase), TSR(Tartronic semialdehyde reductase)



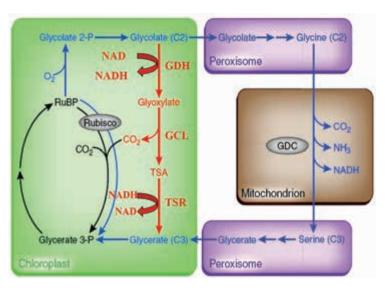


Fig. 4. Proposed diagrammatic scheme for introduction of bacterial glycolate catabolic pathway in rice chloroplast to bypass photorespiration (GDC: glycine decarboxylase; RuBP: ribulose 1,5-bisphosphate; RuBisCO: ribulose 1,5-bisphosphate carboxylase-oxygenase; GDH: glycolate dehydrogenase; GCL: glyoxylate carboxyligase; TSA: tartronic semialdehyde reductase).

### 4. CHALLENGES

It is certainly a difficult job to achieve a similar level of photosynthetic efficiency of C<sub>4</sub> plants by transferring C<sub>4</sub> specific genes in C<sub>3</sub> plant. Lots of metabolic and anatomical differences exist between C<sub>3</sub> and C<sub>4</sub> plants. Transferring the C<sub>4</sub> metabolism and anatomy to rice is undoubtedly one of the most ambitious crop engineering approaches ever. There is no simple rule but coordinated activity of multiple enzymes in different cell types in response to diverse environmental and metabolic stimuli are required for a true C<sub>4</sub> cycle engineering. When plants first evolved, photorespiration was not a problem, because the atmosphere then was high in CO<sub>2</sub> and low in O<sub>2</sub>. As a byproduct of photosynthesis, O2 accumulated in the atmosphere and reached the present level. Unluckily, current atmospheric CO2 levels limit photosynthesis in C3 plants, hence, due to its better mechanism C4 plants are able to maintain their productivity even in adverse climatic condition. The introduction of C<sub>4</sub> photosynthesis into C<sub>3</sub> species requires major changes in leaf anatomy and various genes required for enzymatic reaction. For the development of peculiar subcellular anatomy, the genes are unknown. Considering the lack of information of single cell C<sub>4</sub> species, it is difficult to bioengineer  $C_4$  metabolism in to  $C_3$  crop.

460



### 5. WAY FORWARD

Although  $C_4$  leaves have close veins and high rates of photosynthesis,  $C_4$  photosynthesis is also naturally supported around widely spaced veins in maize husk tissue, albeit at lower rates. Thus, a prototype  $C_4$  rice may be achievable with a subset of  $C_4$  genes, but a good  $C_4$  rice will require substantial fine tuning of biochemistry and anatomy. Particularly intriguing is the need for additional metabolite transport across membranes of organelles in  $C_4$  photosynthesis. A functional  $C_4$ -concentrating mechanism in rice would allow for an approximately two-third reduction in Rubisco levels, relative to wild-type rice, but Rubisco would be sequestered in bundle sheath cells and ideally have a greater catalytic turnover rate. Antisense gene suppression of key photosynthetic enzymes has illuminated  $C_4$  metabolism and engineering strategies including the surprising phosphorylation of PEPC by the regulatory enzyme PEP carboxylase phosphokinase is not needed for  $C_4$  function.

If the attempts to alter the photosynthesis of rice from  $C_3$  to  $C_4$  pathway by introducing cloned genes from maize/sorghum to regulate the production of enzymes responsible for  $C_4$  synthesis are successful, the yield potential of rice of our country may increase by 30-35%. The single cell  $C_4$  photosynthetic system gives us hope to genetically engineer all  $C_4$  genes in single cell of  $C_3$  plants (rice) to enhance its photosynthetic activity and productivity.

#### References

- Busch FA, Sage TL, Cousins AB and Sage RF (2013) C3 plants enhance rates of photosynthesis by reassimilating photorespired and respired CO<sub>2</sub>. Plant Cell & Environ 36: 200-212.
- Chen P, Li X,Huo K, Wei X, Dai C, &Lv C. (2014). Promotion of photosynthesis in transgenic rice over-expressing of maize C4 phosphoenolpyruvate carboxylase gene by nitric oxide donors. J. of Pl. Phyo. 171(6):458-466.
- Chi W, Zhou JS, ZhangF, and Wu NH (2004) Photosynthetic Features of Transgenic Rice Expressing Sorghum C4 Type NADP-ME. Acta Botanica Sinica 46(7): 873-882.
- Gu JF, Qiu M, and Yang JC (2013) Enhanced tolerance to drought in transgenic rice plants overexpressing C4 photosynthesis enzymes. The Crop Journal 1(2): 105-114.
- Huang YR, Wu Y, IzuiK and Ku MS (2008) Genetic Engineering of "C 4 Rice": Expression of Maize PEP Carboxykinase in Rice Mesophyll Chloroplast to Raise CO 2 Concentration and Photosynthesis. Photosynthesis Energy from the Sun pp. 1225-1230.
- IzuiK, MatsumuraH, FurumotoT, and KaiY (2004) Phosphoenol pyruvate carboxylase: a new era of structural biology. Annual review of Plant Biology 55: 69-84.
- Lian L, Wang X, Zhu Y, He W, Cai Q, Xie H and Zhang J (2014) Physiological and photosynthetic characteristics of indica Hang2 expressing the sugarcane PEPC gene. Molecular Biology Report 41(4): 2189-2197.
- Miyao M (2003) Molecular evolution and genetic engineering of C4 photosynthetic enzymes. Journal of Experimental Botany 54(381): 179-189.
- Sage RF (2004) The evolution of C4 photosynthesis. New Phytologist 161(2): 341-370.
- Sen P, Ghosh S, Sarkar SN, Chanda P, Mukherjee A, Datta SK and Datta K (2017) Pyramiding of three C4 specific genes towards yield enhancement in rice. Plant Cell Tissue and Organ Culture 128(1): 145-160.



- Tsuchida H, Tamai T, Fukayama H, Agarie S, Nomura M, Onodera H and Toki S (2001) High level expression of C4-specific NADP-malic enzyme in leaves and impairment of photoautotrophic growth in a C3 plant, rice. Plant and Cell Physiology 42(2):138-145.
- Wang J and Li R (2008) Integration of C4-specific ppdk gene of Echinochloa to C3 upland rice and its photosynthesis characteristics analysis. African Journal of Biotechnology 7(6): 783-787.
- Wang L, Deng F and Ren WJ (2015) Shading tolerance in rice is related to better light harvesting and use efficiency and grain filling rate during grain filling period. Field Crops Research 180: 54-62.
- Xue W, Lindner S, Nay-Htoon B, Dubbert M, Otieno D, Ko J, Muraoka H, Werner C, Tenhunen, J. and Harley P (2016) Nutritional and developmental influences on components of rice crop light use efficiency. Agricultural and Forest Meteorology 223: 1-16.
- Yang XC and Hwa CM (2008) Genetic modification of plant architecture and variety improvement in rice. Heredity 101(5): 396-404.
- Yuan LP (1997) Hybrid rice breeding for super high yield. Hyb. Rice 12: 1-6.
- Zhang J, Bandyopadhyay A, Sellappan K, Wang G, Xie HA, Datta K and Datta SK (2010) Characterization of a C4 maize pyruvate orthophosphate dikinase expressed in C3 transgenic rice plants. African Journal of Biotechnology 9(2): 234-242.
- Zheng B, Shi L, Ma Y, Deng Q, Li B and Guo Y (2008) Comparison of architecture among different cultivars of hybrid rice using a spatial light model based on 3-D digitising. Functional Plant Biology 35(10): 900-910.\*



## Abiotic Stress Tolerance in Rice: Physiological Paradigm under Changing Climatic Scenario

### P Swain, MJ Baig, K Chakraborty, N Basak, PK Hanjagi, SK Pradhan, A Anandan, NP Mandal, K Chattopadhyay, JL Katara and G Kumar

### **SUMMARY**

Grown under diverse range of ecosystems, rice gets exposed to different environmental stresses like drought, salinity, submergence, cold as well as high temperature and lowlight. In the era of global climate change, rice cultivation especially in the rain-fed ecology (rainfed upland, shallow lowland and lowland) faces multifacet problems. The changing climatic conditions make the rice crop vulnerable to moderate to severe drought stress, germination stage oxygen deficiency (GSOD) or submergence stress depending upon the timing of the natural events, and increase in salinity level in coastal rice belts, lowlight stress situation due to heavy down pour or prolonged cloudy weather conditions and heat stress due to increase in temperature during dry spells mostly in grain filling period. Recent reports suggest more frequent occurrence of such climatic extremities in many parts of the Indian subcontinent and elsewhere. Yield improvement in such stressful environments could be achieved by identifying secondary traits contributing tolerance to a particular stress or combination of stresses and selecting for those traits in breeding programme. Land races, one of the important components of the germplasm, serve as donors for different abiotic stress tolerance and have broad genetic base which provides them wider adaptability and protection from various stresses. So, identification of suitable donor and secondary/putative traits for developing high yielding varieties through conventional or molecular approaches with an added advantage of understanding stress tolerance mechanism is of paramount importance in the present scenario.

### **1. INTRODUCTION**

Rice is grown in different ecologies covering about 44.0 m ha throughout India. Due to variations in geographic situations and rainfall pattern, the country experiences different abiotic constraints. Climate change and irregularities in South-west monsoon result in moderate to severe droughts in rainfed rice growing areas, submergence/ waterlogging even during reproductive stage in low lying areas, elevated temperature regimes during both vegetative and reproductive stages and different intensities of low light during the cropping period mostly in eastern Indian states.

Crop yield depends on specific climatic conditions and is highly affected by climate variations. Estimation of the overall rice yield variation due to climate variability over the last three decades showed that approximately 53% of rice harvesting regions experiences the influence of climate variability on yield at the rate of about 0.1 t/ha per year and approximately 32% of rice yield variability is explained by year-to year global climate variability (Fig. 1).



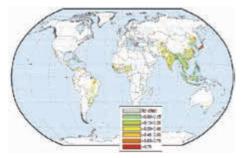


Fig. 1. Global rice yield variability due to climate variability over the last three decades (Ray et al. 2015).

The objectives of this chapter are to outline the major abiotic stresses that affect rice production and to understand the tolerance mechanism of each.

#### 1.1. Drought

Water is an important factor in agricultural and food production and yet is a highly limited resource. Water deficit stress causes extensive loss to agricultural production worldwide, thus being a severe threat to

sustainable agriculture. Out of 44.0 million ha area under rice in India, drought is one of the major abiotic constraints in around 8.0 million ha of rainfed upland and rainfed lowland situations. About 18% of total rice area of India and 20% of Asia are drought prone. The irregularities in south-west monsoon do result in moderate to severe drought in rainfed rice growing areas especially in eastern India. Drought is a multifaceted stress condition with respect to timing and severity, ranging from long drought seasons where rainfall is much lower than demand, to short periods without rain where plants depend completely on available soil water (Lafitte et al. 2007). Among the different environmental stresses, drought constitutes an important yield limiting determinant. Food security and prosperity of India is challenged by increasing demand and threatened by declining water availability thereby requiring crop varieties that are highly adapted to dry environments.

#### 1.2. Submergence

Submergence is a type of flooding stress, which is defined as a condition, where the entire plant is fully immersed in water (a phenomenon termed as complete submergence) or at least part of the shoot terminal is maintained above the water surface (a phenomenon termed as partial submergence). Under submergence, plants face a number of external challenges simultaneously or sequentially, which results in multiple internal stresses that affect plant growth and survival. Submergence substantially reduces the gas diffusion rate in the leaf tissue, restricting oxygen uptake and forcing carbon inefficient carbohydrate metabolism *via* anaerobic route (Panda et al. 2017). To add-on to the problem, turbid floodwaters also reduce light availability, inhibiting underwater photosynthesis and leaf gas exchange. Limitation of efficient gas exchange also restricts transpiration severely, possibly impeding the absorption and transport of nutrients from the soil.

#### 1.3. Salinity

Soil salinization is a worldwide problem for agriculture affecting 6% of total Earth's land, as a result of natural accumulation over long periods of time. However, agricultural activity contributes to secondary salinization: 2% of all dry land is becoming salinized, and more than 20% of irrigated soils are affected, mostly because of irrigation water



containing small amounts of sodium chloride. Plants do vary in their sensitivity to salinity stress. Although being the most sensitive amongst all the cereals, having a threshold salinity level of only 3 dS m<sup>-1</sup>, rice show considerable variability across its different species and also within different genotypes of the same species (Menguer et al. 2017).

#### 1.4. High temperature

The constantly rising temperature is one of the most detrimental problem profoundly affecting plant metabolism. Heat stress in rice affects the anthesis and grain filling stages of the crop. Even one degree rise in temperature above the optimum temperature results in 7-10% loss in the yield of rice (Fahad et al. 2017). Apart from yield, heat stress also has a negative influence on various grain quality traits and reduces the sensory attributes. As the eastern part of India experiences early sunrise, temperature often rise above 35°C during the time of anthesis in the months of April and May. Consequently, late sown rabi season rice crop and timely sown long duration cultivars are highly sensitive to elevated temperature stress in eastern India.

#### 1.5. Lowlight

The low incidence of solar radiation coupled with fluctuating light due to overcast sky during the wet season is one of the major constraints for realizing the low productivity in eastern and northeastern India. It induces high tiller mortality at vegetative stage, reduction in spikelet number at reproductive stage and dry matter production after flowering which drastically affects production. Rice yield during the wet season is 65.2% of that of dry season. Rice that receives half of the sunshine has 27-37% less yield than rice receiving full sunshine (Murty et al. 1992). The future increase in production has to come from these neglected regions as they harbour huge area with low productivity. Tolerance to low light is genetically controlled, but until recently little has been known about the genes involved. In order to design rice genotypes with higher yield and greater stability under low light stress, evaluation of rice germplasms tolerant to low light conditions is required along with a systematic investigation of the mechanisms of tolerance to light stress.

## 2. PHYSIOLOGICAL AND BIOCHEMICAL BASIS OF DROUGHT TOLERANCE

Though rice is a water loving plant, yet it can successfully be grown under upland ecosystem due to its adaptability to low moisture conditions. However, its productivity is much lower than what we get in irrigated/lowland ecologies. Drought tolerance is a complex trait, which is a combined function of various morphological, biochemical and molecular characters. Knowledge about these characters that maintain plant growth and development during water stress conditions is paramount in understanding stress response processes. Morphological traits viz., maintenance of turgor, initiation of leaf rolling, cuticular wax, deep and coarse root with greater xylem vessel radii and lower axial resistance to water flux are indicators of drought tolerance.





Most physiological and metabolic processes are affected by water deficits which include stomatal regulation, photosynthesis, translocation, PSII activity, chlorophyll content, etc. Maintenance of these processes for prolonged period of time under drought is a desired character. Since, ABA is an important component of signalling under drought stress, efficient ABA signalling also ensures tolerance. Biochemical parameters viz., proline and polyamine accumulation in plants increases under drought stress. Further, the enhancement of the naturally occurring antioxidant components (enzymatic and non-enzymatic) may be another strategy for reducing oxidative damage and can be considered to be a vital mechanism of drought tolerance (Pandey and Shukla 2015). In addition, a very large number of genes in rice are up- or downregulated by drought which not only enhances the plant survival in drought conditions but also improves the crop productivity. Recently, many transcription factors (TFs) have been identified in rice, the expression of which provides drought tolerance as well as improves yield under stressful conditions. To facilitate the selection or development of drought tolerant rice varieties, a thorough understanding of the various mechanisms that govern the yield of rice under water stress condition is a prerequisite.

## 3. PHYSIOLOGICAL AND BIOCHEMICAL BASIS OF HEAT TOLERANCE

Rice is highly sensitive to heat stress particularly at flowering and post-flowering stages. Exposure to short periods of heat stress coinciding with flowering has resulted in significant yield losses in India, China and Japan. During anthesis heat stress leads to irreversible reduction in spikelet fertility mainly by affecting sensitive physiological processes such as anther dehiscence, pollination, and early fertilization events. To minimize heat stress damage plants generally adopt three mechanisms *viz.*, heat escape (time of day of flowering, especially early morning flowering), heat avoidance through transpirational cooling and heat tolerance through resilient reproductive processes (Jagadish et al. 2010). High temperature may also hamper proper functioning of the enzymes of nitrogen uptake and assimilation and also photosynthesis. Decline in the activities of source and sink also significantly affects the growth and eventually the economic yield of rice.

## 4. PHYSIOLOGICAL AND BIOCHEMICAL BASIS OF SUBMERGENCE TOLERANCE

Rice plants tolerant to complete submergence usually exhibit very limited elongation during submergence and often show tolerance to complete flooding, a strategy known as quiescence. Some researchers believe that the ideal combination for adaptation to complete flooding is submergence tolerance (survival under water) together with some elongating ability. Under water photosynthesis and utilization of existing carbohydrate reserve is found to be one of the most important factors for submergence tolerance in rice (Das et al. 2009). Studies have shown that the differences in tolerance



level were not necessarily associated with the initial carbohydrate status before submergence but with the plant's ability to maintain high levels of stored energy through either slower utilization during submergence and/or greater underwater photosynthesis.

The mechanistic understanding of molecular regulation of submergence tolerance in rice has been advanced through functional characterization of key genes responsible for acclimation to submergence stress in rice (Xu et al. 2006). Limited number of rice genotypes possess inherent mechanism to tolerate a deep transient flash flood through economization of energy reserves (quiescence strategy) (Fukao et al. 2008). Quantitative trait locus (QTL) analysis and map-based cloning revealed that the SUBMERGENCE1 (SUB1) locus, encoding a variable cluster of two or three tandemrepeated group VII of ETHYLENE RESPONSIVE FACTOR (ERF-VII), regulate the quiescence response (Xu et al. 2006). Most of the reported rice accessions were found to contain SUB1B and SUB1C genes at the SUB1 locus, whereas SUB1A was reported to contribute ~70% of submergence tolerance to some *indica* and *aus* rice varieties (Singh et al. 2010).

## 5. PHYSIOLOGICAL AND BIOCHEMICAL BASIS OF SALINITY TOLERANCE

Generally, salinity causes two types of stresses on plants: osmotic and ionic stresses. The genetic basis of tolerance to ionic stress is much better understood than that of osmotic stress. Between the two main sub-species of rice, it is observed that *indica* is more tolerant than *japonica*. Tolerant *indica* varieties are good Na<sup>+</sup> excluders, absorb high amounts of K<sup>+</sup>, and maintain a low Na<sup>+</sup>/K<sup>+</sup> ratio in the shoot.

Sodium, an integral constituent of our earth crust is naturally present in all soil types. At lower concentration Na<sup>+</sup> may promote growth in rice but eventually it becomes toxic when present in high concentration in growing medium. Both Na<sup>+</sup> and K<sup>+</sup> share high similarity in ionic as well as its physicochemical properties, but unlike Na<sup>+</sup>, K<sup>+</sup> are integral part of plant's life and play essential role in growth and development. Many basic physiological processes, which are essentially dependent on K<sup>+</sup> shows impairment due to hindrances in specific transport and interactions of K<sup>+</sup> with enzymes and membrane proteins (Britto and Kronzucker 2008). This may well be of diverse role *viz*. short-term maintenance of membrane potentials to pollen tube development and stomatal opening and closing (Dietrich et al. 2001).

Initially osmotic stress occupies the main position whereas with time more salt is absorbed by the plant and ionic stress plays the leading role. Adaptation to salt stress is to adjust with both osmotic and ionic stresses. Salt stress is cumulative. With time, injury symptoms increase. Susceptible cultivars die early compared to tolerant cultivars in a salty environment. So, restricting the movements of ions such as Na<sup>+</sup> and Cl<sup>-</sup> to growing meristematic tissues and young photosynthetic organs are crucial for survival. Tolerant rice cultivars like Pokkali either absorbs low levels of Na<sup>+</sup> or restricts the movement of Na<sup>+</sup> in comparison to K<sup>+</sup> and thereby maintains low



Na<sup>+</sup>:K<sup>+</sup> ratio in shoot/leaf and protects the vital tissues (Kobayashi et al. 2017). High Na<sup>+</sup> in the cell cytoplasm impairs several physiological and biochemical courses of action which restricts plant growth.

## 6. MECHANISM FOR LOWLIGHT TOLERANCE

Little is known about the mechanisms of low light tolerance in rice crop in terms of physiological and molecular scale. When plants are shaded, the photo-assimilates that fuel growth become limited; the growth of the stem is promoted to place the leaves at a higher position, with better chances to capture light for photosynthesis. This response involves enhanced growth when the available photo-assimilates are already limiting.

### 7. MOLECULAR MECHANISM OF STRESS TOLERANCE

Drought is a main yield reducing factor for rainfed rice. The progress of rice improvement is very slow. However, few yield QTLs under drought at reproductive stage are valuable research advancement in drought breeding. As per the mapping results, the yield QTLs qDTY1.1, qDTY2.1 and qDTY3.1 are effective under drought stress (Dixit et al. 2014). Amongst these, *qDTY1.1* was a consistent QTL showing effect on per se yield under drought affected lowland ecology. The other two QTLs also showed higher phenotypic variances for yield under drought situation. For upland ecology, a QTL, *qDTY12.1* has been detected showing very high additive effects and found to be effective in drought breeding (Dixit et al. 2014). Another QTL, qDTY3.2 exhibits a positive effect on yield under drought stress. Irrigated rice varieties possess mostly surface spreading type root system. In rainfed farming, these varieties are highly affected by moisture stress situations due to short drought spells. Hence, a deeper rooting system escapes better under such situation. The QTL, Dro1 makes the root to move downward by bending of the root in response to gravity. DEEPER *ROOTING 1 (Dro1)* is a major quantitative trait locus which helps in escaping drought stress. Higher expression of *Dro1* increases the root growth angle, whereby roots grow in a more downward direction (Uga et al. 2013). Introducing Drol into a shallowrooting rice cultivar by backcrossing enables resulting deep rooting recipient cultivar. Variation in root growth angel in rice exists and may be controlled by a few major QTLs and by several additional minor QTLs (Kitomi et al. 2015).

The yield loss due to flash flood is very high in lowland rice ecology. A major QTL (*Sub1*) explaining about 70% of phenotypic variation for submergence tolerance has been identified and fine mapped on chromosome 9 in the submergence tolerant cultivar FR13A (Xu et al. 2000).

## 8. WORK DONE INTERNATIONALLY AND NATIONALLY ON VARIOUS ABIOTIC STRESSES

Drought stress is a very important limiting factor at the initial phase of plant growth and establishment. It affects both elongation and expansion growth (Shao et



al. 2008). A soil water potential threshold of 20 kPa at 30 cm depth during the vegetative stage was identified as the target for effective selection under vegetative stage with grain yield reduction of about 50% compared to irrigated control trials (Swain et al. 2017). Roots are crucial for nutrient and water acquisition and can be targeted to enhance productivity under a broad range of growing conditions. A dynamic root system is fine-tuned to soil moisture status and is known to regulate the amount of water available to the plant depending on its distribution in the soil. Among the root morphological traits, maximum root length, root diameter and root:shoot dry weight ratio were found to be associated with drought resistance in upland conditions. Root thickness was found to confer drought resistance, as roots are capable of increasing root length density and water uptake by producing more and larger root branches (Ingram et al. 1994). Shoot growth is reported to be more inhibited than root growth when soil water is limited. This differential response/sensitivity of root and shoot growth to low-water potential is considered as a means of avoiding excessive dehydration (Sharp and Davies 1989). Increased root:shoot ratio, high total root length and high root elongation rates enable plants to maintain relatively high water uptake (rates) under water stress conditions.

Photosynthetic ability has been regarded as an important indicator of the growth of plants, because of their direct link to net productivity. Drought causes not only a substantial damage to photosynthetic pigments, but it also leads to deterioration of thylakoid membranes. Chlorophyll pigment played an important role in photosynthesis and chlorophyll stability index as a measure of integrity of membrane of the pigments was found to correlate with drought tolerance. The strong relationship between drought susceptibility index (DSI) and percent change in SPAD Chlorophyll Meter Reading (SCMR) under water deficit condition indicate that higher chlorophyll concentration is important for adaptation to water deficit conditions during grain filling period (Talwar et al. 2011). DSI is also negatively related to yield under stress. Several researchers have proposed the use of stomatal conductance (g) as an indicator to assess the difference between stomatal and nonstomatal limitations to photosynthesis under water-limited environments. Regulation of leaf stomatal conductance  $(g_i)$  is a key phenomenon in plants as it is vital for both prevention of desiccation and CO, acquisition. Cultivars that exhibited the highest values of total conductance to CO<sub>2</sub> supported higher photosynthesis and yield under all levels of water availability (Gu et al. 2013).

Proline as an osmo-regulatory solute acts as an osmo-protectant under drought and salinity stress, its concentration increasing in stressed plants due to stimulation of proline biosynthesis. In drought condition, some reactive oxygen species (ROS) are created, and to overcome oxidative stress, plants develop enzymatic and non enzymatic antioxidant defence mechanisms to scavenge ROS (Smirnoff 1993). The most important antioxidant enzymes are super oxide dismutase (SOD), catalase (CAT) and peroxidase (POD). SOD converts  $O^{2-}$  into  $H_2O_2$  and  $O_2$ , while CAT and POD scavenge  $H_2O_2$  into  $H_2O$  (Wang et al. 2009).



Significant genotypic variation has been documented in rice for heat stress induced spikelet sterility. Variability in heat stress induced spikelet sterility has been explained by air temperature, and interaction effect of air temperature and relative humidity on plant tissue temperature. Large differences between the panicle and air temperature, primarily due to vapor pressure deficit which is a function of prevailing temperature and relative humidity (RH) have been reported (Yoshimoto et al. 2012). A recent study showed that the effects of heat stress on sterility were due to the high temperatures recorded in the organ itself and not that of the environment. Rice germplasms for high temperature stress phenotyping to quantify the relationship between spikelet sterility and air, leaf, panicle and canopy temperature in order to develop thermo tolerant rice genotypes.

Internationally, the focus has also been on assessing quality parameters of rice as affected by heat stress. It has been suggested that the temperature during grain filling is an important factor influencing grain quality. It is seen that rice plants when grown under high temperature have low amylose content compared to those grown under low temperatures. It has been reported that high ambient temperatures in later stages of development are responsible for reduction in spikelet fertility (Jagadish et al. 2010) and reduced grain quality as the endosperm becomes chalky in texture (Fitzgerald et al. 2009). Elevated temperatures during growing period have also been shown to cause alterations in important physicochemical properties of rice starches related to food processing quality. Studies report that high-temperature stress suppressed the expression of the starch-synthesis-related genes *GBSSI, BEIIb, SuSy2*, and *AGPS2b* to about 50 and 80% of that in the control conditions throughout grain filling. Hakata et al. (2012) also reported that activation of  $\alpha$ -amylase by high temperature was a crucial trigger for grain chalkiness.

Importance of aerenchyma under long-term water-logging is well established and studies showed that the short-term (7 days) exposure of rice plants to complete submergence induced the formation of aerenchyma tissues in roots, a process much faster in tolerant (FR13A) than in susceptible (IR42) genotypes (Nishiuchi et al. 2012). Rice being a wetland crop is somewhat tolerant to anaerobiosis and such submergence induced adaptive traits *viz*. aerenchyma formation and narrower leaves and leaf mass/ area probably helps them to withstand the ill-effect of anoxia during submergence stress.

The variety Swarna possessing *Sub1* has become very popular in rainfed lowland ecologies in the country. Swarna-Sub1 was released by SVRC, Odisha and SVRC, Uttar Pradesh and notified by Dept. of Agriculture and Cooperation, Ministry of Agriculture, Govt. of India.

Rice has been reported to be relatively tolerant to salinity stress during germination, active tillering and towards maturity, but sensitive during early seedling and reproductive stages, where an addition of as little as 50 mM NaCl in the soil can reduce rice yield significantly (Zeng et al. 2003). A range of transporters involved in

470



reducing  $\mathrm{Na^{\scriptscriptstyle +}}$  accumulation in shoots and in sub-cellular compartmentalization was identified.

The high affinity potassium transporter (HKT), salt overly sensitive (SOS) and Na<sup>+</sup>/H<sup>+</sup>Exchanger (NHX) gene families are key players imparting salt tolerance in rice. The HKT members are crucial determinants of tissue concentration of Na<sup>+</sup>. *OsHKT1;5* was identified as the causative gene of *Saltol*, the major quantitative trait locus (QTL) for salt accumulation in *O. sativa* genotypes (Ren et al. 2005). OsHKT1;5 is a plasma membrane transporter that regulates partitioning of Na<sup>+</sup> between roots and shoots by efflux of Na<sup>+</sup> from the xylem to adjacent parenchyma cells. Robust screening effort including several *O. sativa* cultivars, landraces and *O. glaberrima* (AA genome) genotypes, showed that salinity sensitivity is correlated with Na<sup>+</sup> concentration in the leaf blades. OsHKT1; 5 genotype was shown to be a major determinant for tolerance: the more active the efflux transporter, which directs the Na<sup>+</sup> exclusion from the transpiration stream, the less Na<sup>+</sup> is translocated to leaves.

Researchers across the world are trying to explore other species from the genus *Oryza* for salt tolerance genes. *O. rufipogon* was shown to be salt tolerant when compared to rice sensitive cultivars. Introgression lines derived from *O. rufipogon*×*O. sativa* cross revealed 15 QTLs for salinity tolerance, 13 of which were derived from the *O. rufipogon* parent (Tian et al. 2011). Over-expression of bHLH transcription factors OrbHLH001 and OrbHLH2 from *O. rufipogon* resulted in *Arabidopsis* and *O. sativa* salt tolerant lines (Chen et al. 2013). It was found that OrbHLH001 is able to positively regulate the K<sup>+</sup> transporter *OsAKT1*, suggesting that salt tolerance results from maintenance of K<sup>+</sup> homeostasis under high Na<sup>+</sup> conditions (Chen et al. 2013).

At national level, very few discrete attempts had been made to compare the relative salt tolerance capacity of rice genotypes of different ecologies of Indica type rice genotypes belonging to *O. sativa* (Omisun et al. 2017). Salt tolerance in native genotypes of north eastern India was attributed to efficient action of Na<sup>+</sup>/K<sup>+</sup> co-transporters, OsHKT2;1, OsHKT2;3 and OsHKT2;4, which is a predominant salinity tolerance mechanism in *O. sativa* (Omisun et al. 2017).

## 9. WORK DONE AT NATIONAL RICE RESEARCH INSTITUTE

To identify rice germplasm lines with built-in tolerance to vegetative and reproductive stage drought, large number of rice germplasm including upland rice, lowland rice, deep water rice, wild rice, aromatic rice and fixed lines are being screened at ICAR-NRRI, Cuttack under field condition during dry season. Generally for large scale screening, experiments are conducted under field condition during dry season where interference of rain is negligible during the cropping period. Wherever controlled facility like rain-out shelter is available, screening is done in wet (kharif) season also (Fig. 2). In this process, more than 10,000 germplasms were screened and a good number of genotypes (>250) for vegetative and (>50) for reproductive stage

471





Fig. 2. Screening for drought tolerance under field and rainout shelter.

drought stress tolerance already have been identified in our institute. Ten genotypes were identified tolerant to intermittent drought spells in rainfed upland conditions yielding >2.0 t ha<sup>-1</sup> with lower spikelet sterility (9.0–14.7%).

During evaluation for vegetative stage drought tolerance, morpho-physiological changes viz. leaf rolling and death score based on standard evaluation system, plant water status like Relative Water Content (RWC), Leaf Water Potential (LWP), membrane stability index, water use efficiency and transpiration, chlorophyll content and chlorophyll stability index, proline content, gas exchange parameters, chlorophyll fluorescence, photosystem II yield, phenotyping for root morphological traits, Water Use Efficiency (WUE) etc. are phenotyped. Better plant vigour, total protein content, catalase and peroxidase activity under mild osmotic stress was observed in tolerant genotypes AC 42994, AC 43030 and AC 43012.

Evaluations for key adaptive traits for vegetative and reproductive stage drought tolerance are made in different experiments in field and controlled environments. Genotypes maintaining high turgidity during severe stress (RWC>70%) and recovered faster on re-irrigation had higher efficiency for drought tolerance. Significant negative correlation between drought score vs. RWC (r= -0.78\*) and drought score vs.  $F_v/F_m$  (r= -0.610\*) and significant positive correlation between RWC and  $F_v/F_m$  (r=0.710\*) indicates that plants having higher water content are able to harvest most of the photon falling on the canopy and radiate less energy in the form of fluorescence (Fig. 3).

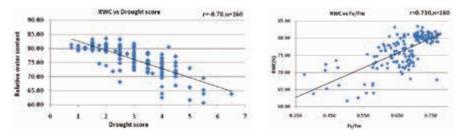


Fig. 3. Correlation of relative water content with drought score and Fv/Fm.



The root:shoot ratio, maximum root length to shoot length ratio and root volume were observed to be the most crucial morphological markers in determining drought tolerance in rice genotypes analyzed through biplot analysis. Among the thirteen genotypes tested, AC-42994, AC-42997, AC-43020, CR-143-2-2, Ronga Bora and Bora were found to possess desirable root traits and these genotypes can be used in the breeding programme for enhancing drought tolerance in rice (Dash et al. 2017).

For reproductive stage stress tolerance, phenology, spikelet fertility/grain filling per cent, yield and its components, relative yield reduction and drought susceptibility index etc. used to be considered as important traits. Genotypes BVD-109 (2.15 t ha<sup>-1</sup>), Kalakeri (2.08 t ha<sup>-1</sup>), IC 416249 (2.02 t ha<sup>-1</sup>) and CR 143-2-2 (1.90 t ha<sup>-1</sup>), AC 27675, IC 516130, Udayagiri, Indira, Vandana , AC-42994, Brahman-nakhi, AC-43006, Nania Kalabora, EC 545088 and IC 337606 have been identified as promising having grain yield of >1.5 t ha<sup>-1</sup> with minimum yield reduction (18-45%) and low DSI (<0.65) at soil moisture content of 9.8-13.3%.

Drought Susceptibility Index (DSI) was identified as a selection criteria for yield under stress. The mean values of DSI close to or below one for grain yield (GY) in the genotypes indicated their relative tolerance to drought (Fig. 4).



Fig. 4. Correlation between grain yield and DSI under control and stress conditions.

Under 55% field capacity, CR 143-2-2 (tolerant check) along with AC-43025, AC-43037 and AC-42997 had highest water use efficiency coupled with slow transpiration rate above VPD 5 kPa, low stomatal density with lower canopy temperature maintenance ( $34.02 \degree C - 41.18 \degree C$ ) (Fig. 5) leading to higher biomass production using less water compared to susceptible check IR 64 (Dash et al. 2015).

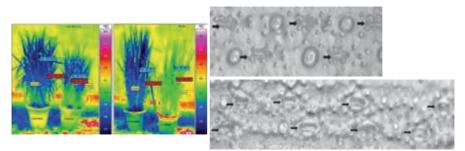


Fig. 5. Canopy temperature and stomatal density in two contrasting genotypes.



Screening work carried out at NRRI identified three out of 30 advance breeding lines CR 3564-1-2-4-1-1, CR 3564-1-1-1-1 and CR 3581-1-1-1-1 and four out of 42 germplasm lines AC 39834, AC 39843, AC 39969 and AC 4391 as best heat tolerant lines over 3 years with >80% spikelet fertility. Quality analysis of the heat stressed samples were carried out and it was found under high temperature stress, amylose content was high, gel consistency was low, alkali spreading value and grain breadth did not change much, while length and L/B ratio reduced by 16% and 14%, respectively as compared to ambient temperature.

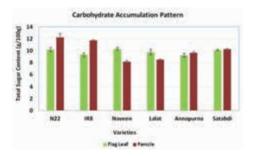


Fig. 6. Carbohydrate accumulation pattern in flag leaves and panicles of tolerant and susceptible varieties.

The effect of high temperature on grain yield and carbohydrate accumulation was studied in seven contrasting genotypes (N22, Ratna, Annapurna, Satabdi, IR72, Lalat and Naveen) grown in field at four different dates of sowing at 10 days interval. Grain yield was reduced under high temperature stress to the tune of 43.7 to 47.9% in susceptible varieties like Naveen and Satabdi, while tolerant variety Annapurna and N22 showed very minimal 6.9 to 18.3% of reduction.

Accumulation of total sugar was reported to be very high in tolerant variety N22 as compared to susceptible varieties suggesting the impaired carbohydrate mobilization process due to high temperature stress (Fig. 6).

Significant progress has been made in submergence studies in rice at ICAR-NRRI, Cuttack. The genotype, FR13A (vernacular name 'Dhalaputia', Odisha, India) was identified from this institute as a true submergence tolerant genotype. FR13A is the source of submergence tolerance gene/QTL (SUB1) which imparts submergence tolerance. Extensive work was done with this genotype and mechanism of submergence tolerance is now fairly understood. In other words, it can be said that NRRI (then CRRI) and Odisha presented the SUB1 gene to the rest of the world. Genotypes with SUB1 can withstand complete submergence depending on flood-water characteristics up to 1-2 weeks. One of the key finding for submergence tolerance of rice identified from here is that non-structural carbohydrates content before and after submergence is important for providing energy for maintenance of key metabolic processes during submergence and for regeneration and recovery of seedlings after submergence (Panda et al. 2017). Studies have shown that the differences in tolerance level were not necessarily associated with the initial carbohydrate status before submergence but were rather associated with the plant's ability to maintain high levels of stored energy through either slower utilization during submergence and/or greater underwater photosynthesis. The initial carbohydrate level before submergence was almost equal in rice cultivars Gangasiuli and Raghukunwar, but Gangasiuli showed better survival



percentage (51%) than Raghukunwar (36%), with retention of higher chlorophyll and non-structural carbohydrate contents during submergence.

The diversity in microsatellite markers in the *Saltol*-QTL region among 30 accessions from saline tracts was examined and validated by using 37 breeding lines that were salt tolerant at the seedling stage and the diversity was assessed in terms of morpho-physiological traits related to salt stress (at 12 dS m<sup>-1</sup>) which showed moderately tolerant nature of the accessions collected from coastal areas in two Indian states, West Bengal and Odisha and were distant from a *Saltol*-introgressed line, namely FL478 (Chattopadhyay et al. 2014). Singh and Sarkar (2014) reported chlorophyll fluorescence characteristics for distinction and characterization of salinity tolerant and sensitive rice cultivars effective for Indian rice cultivars. Probing with chlorophyll fluorescence technique, Sarkar and Ray (2016) reported, submergence-tolerant rice (FR13A in this case) can withstand complete submergence even in saline water and significantly longer duration of time. Chattopadhyay et al. (2014) reported salt tolerant nature of genotype SR 26B, despite the most distant genotype from Pokkali (known salt tolerant genotype) in the *Saltol* QTL region.

### **10. KNOWLEDGE GAP**

In spite of extensive studies, there is still a strong need for more detailed characterization of the response and acclimation mechanism of rice under drought that is occurring in farmers' fields. Less reduction in grain yield during drought is the critical trait that plays an important role in tolerance against drought. Thus, yield stability under drought conditions and increased crop water productivity should be the target of all the approaches involved in drought tolerance. Molecular attributes are key traits linked to yield under drought.

Heat stress by adversely affecting plant growth and development presents a major challenge in today's scenario. Evaluation of rice germplasms for high temperature stress tolerance employing field based high temperature stress phenotyping to quantify the relationship between spikelet sterility and air, leaf and panicle temperature in the panel of germplasms under study and to identify high temperature stress tolerant genotypes needs to be carried out. In addition, understanding the source-sink relationship of structural and non-structural carbohydrates is required.

From the previous studies, it has been well documented that Sub-1 QTL accounts for as high as 70% of the submergence tolerance in rice. But, there are a few rice genotypes which can withstand submergence stress beyond 14 days. The mechanism thereof needs to be identified. Also, researchers are trying to combine submergence and anaerobic germination in rice to make rice cultivars tolerant to multiple abiotic stresses suitable to rainfed low land ecologies. But, based on the current research outcome, the mechanism of tolerance in these two stresses are almost opposite to each other. So, how these genotypes would be working in case of multiple abiotic stress situations need to be investigated.





Direct seeded rice suffers germination loss due to heavy rainfall in poorly drained soils. Eastern India usually gets severe rains and cyclonic storm during grain maturity stage. Therefore, tolerant QTLs like qAG9.2 and qAG7.1 for anaerobic germination need to be pyramided in the mega variety. In addition, seed dormancy of at least one week should be in the mega variety to avoid vivipary germination.

Although very good progress has been made in the area of salinity tolerance in rice, but a lot of questions still remains unanswered. The ionic basis of  $Na^+/K^+$  homeostasis and the role of ion specific transporter in different plant parts remains elusive. Also, the mechanistic differences in salt tolerance in vegetative and reproductive stages in rice need to be worked out in detail in order to develop salt tolerant rice cultivars particularly tolerant to reproductive stage salinity stress.

Screening for contrasting germplasm lines for low light tolerance will help to develop mapping populations to identify quantitative trait loci that can be used in marker assisted breeding program for tailoring rice varieties. Analysis of molecular processes together with engineering a new light receptor (modified phytochrome) will mitigate the stress as well as reveal mechanisms to reduce the energy cost of light stress in rice.

### **11. CONCLUSION AND WAY FORWARD**

Higher production from abiotic stress situation is essential in this era of climate change and thus poses a challenge to understand the adaptive mechanism and tailor rice genotypes for optimum performance from limited use of resources.

The rainfed rice cultivation in the country is highly affected by the effects of climate change. For this, it is essential to integrate crop physiology, molecular genetics and breeding approaches to dissect complex abiotic stress tolerance traits, and develop the next generation crops which can withstand the adverse climate and ensure food security.

The high yielding varieties should be stacked with stress tolerant gene(s)/QTLs for making them climate resilient. Thus, in mega varieties, multiple tolerant genes for submergence, anaerobic germination, yield QTLs under drought, seed dormancy and yield enhancing QTLs under drought need to be stacked to make them highly resilient to the climate change.

#### References

- Britto DT and Kronzucker HJ (2008) Cellular mechanisms of potassium transport in plants. Physiologia Plantarum 133(4):637-650.
- Chattopadhyay K, Nath D, Mohanta RL, Bhattacharyya S, Marndi BC, Nayak AK, Singh DP, Sarkar RK and Singh ON (2014) Diversity and validation of microsatellite markers in'Saltol'QTL region in contrasting rice genotypes for salt tolerance at the early vegetative stage. Australian Journal of Crop Science 8(3):356-362.



- Chen Y, Li F, Ma Y, Chong K and Xu Y (2013) Overexpression of *OrbHLH001*, a putative helixloop-helix transcription factor, causes increased expression of *AKT1* and maintains ionic balance under salt stress in rice. Journal of Plant Physiology 170(1):93-100.
- Das KK, Panda D, Sarkar RK, Reddy JN and Ismail AM (2009) Submergence tolerance in relation to variable floodwater conditions in rice. Environmental and Experimental Botany 66 (3):425–434.
- Dash GK and Swain P (2015) Differential response of rice genotypes to mild and severe osmotic stress during seedling stage. Oryza 52(4):307-312.
- Dash GK, Barik M, Debata AK, Baig MJ and Swain P (2017) Identification of most important rice root morphological markers in response to contrasting moisture regimes under vegetative stage drought. Acta Physiologia Plantarum 39(8):1-13.
- Dietrich P, Sanders D and Hedrich R (2001) The role of ion channels in light dependent stomatal opening. Journal of Experimental Botany 52(363):1959-1967.
- Dixit S, Singh A, Sta Cruz MT, Maturan PT, Amante M and Kumar A (2014) Multiple major QTL lead to stable yield performance of rice cultivars across varying drought intensities. BMC Genetics 15:16.
- Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S and Ihsan MZ (2017) Crop production under drought and heat stress: plant responses and management options. Frontiers in Plant Science 8(1147):1-16.
- Fitzgerald MA and Resurreccion AP (2009) Maintaining the yield of edible rice in a warming world. Functional Plant Biology 36(12):1037-1045.
- Fukao T and Bailey-Serres J (2008) Submergence tolerance conferred by *SUB1A* is mediated by *SLRI* and *SLRLI* restriction of gibberellin responses in rice. Proceedings National Academy of Science USA 105(43):16814–16819.
- Gu JF, Qiu M and Yang JC (2013) Enhanced tolerance to drought in transgenic rice plants overexpressing  $C_4$  photosynthesis enzymes. The Crop Journal 1(2):105–114.
- Hakata M, Kuroda M, Miyashita T, Yamaguchi T, Kojima M, Sakakibara H and Yamakawa H (2012) Suppression of α-amylase genes improves quality of rice grain ripened under high temperature. Plant Biotechnology Journal 10(9):1110–1117.
- Ingram KT, Bueno FD, Namuco OS, Yambao EB, Beyrouty CA (1994) Rice root traits for drought resistance and their genetic variation. In: *Rice roots: Nutrient* and *water use*. Krik *GJD* (*ed.*), International Rice Research Institute, Philippines, 67-70.
- Jagadish SVK, Cairns J, Lafitte R, Wheeler TR, Price AH and Craufurd PQ (2010) Genetic analysis of heat tolerance at anthesis in rice. Crop Science 50(5):1633-1641.
- Kitomi Y, Kanno N, Kawai S, Mizubayashi T, Fukuoka S and Uga Y (2015) QTLs underlying natural variation of root growth angle among rice cultivars with the same functional allele of DEEPER ROOTING 1. Rice 8(16):1-12.
- Kobayashi NI, Yamaji N, Yamamoto H, Okubo K, Ueno H, Costa A, Tanoi K, Matsumura H, Fujii-Kashino M, Horiuchi T, Nayef MA, Shabala S, An G, Ma JF, Horie T (2017) OsHKT1;5 mediates Na<sup>+</sup> exclusion in the vasculature to protect leaf blades and reproductive tissues from salt toxicity in rice. The Plant Journal 91(4):657-670.
- Lafitte HR, Yongsheng G, Yan S and Li ZK (2007) Whole plant responses, key processes, and adaptation to drought stress: the case of rice. Journal of Experimental Botany 58(2):169–175.
- Menguer PK, Sperotto RA and Ricachenevsky FK (2017) A walk on the wild side: Oryza species as source for rice abiotic stress tolerance. Genetics and Molecular Biology 40(1):238-252.

477



- Murty KS, Dey SK, Swain P and Baig MJ (1992) Low light adapted restorers of different maturity durations for hybrid rice breeding. International Rice Research Newsletter 17(6):6-7.
- Nishiuchi S, Yamauchi T, Takahashi H, Kotula L, Nakazono M (2012) Mechanisms for coping with submergence and waterlogging in rice. Rice 5(2):1–14.
- Omisun T, Sahoo S, Saha B and Panda SK (2017) Relative salinity tolerance of rice cultivars native to North East India: a physiological, biochemical and molecular perspective. Protoplasma 255:193-202.
- Panda D, Rao DN, Das KK and Sarkar RK (2017) Role of starch hydrolytic enzymes and phosphatases in relation to under water seedling establishment in rice. Indian Journal of Plant Physiology 22(3):279-286.
- Pandey V and Shukla A (2015) Acclimation and Tolerance Strategies of Rice under Drought Stress. Rice Science 22(4):147-161.
- Ray DK, Gerber JS, MacDonald GK and West PC (2015) Climate variation explains a third of global crop yield variability. National Communication 6:5989.
- Ren ZH, Gao JP, Li LG, Cai XL, Wei H, Chao DY, Zhu MZ, Wang ZY, Luan S and Lin HX (2005) A rice quantitative trait locus for salt tolerance encodes a sodium transporter. Nature Genetics 37:1141–1146.
- Sarkar RK and Ray A (2016) Submergence-tolerant rice withstands complete submergence even in saline water: Probing through chlorophyll a fluorescence induction OJIP transients. Photosynthetica 54(2):275-287.
- Shao HB, Chu LY, Shao MA, Jaleel CA and Mi HM (2008) Higher plant antioxidants and redox signaling under environmental stresses. Comptes Rendus Biologies 331:433–441.
- Sharp RE and Davies WJ (1989) Regulation of growth and development of plants growing with a restricted supply of water. In: Plants under stress. Jones HG, Flowers TL, Jones MB, (eds.) Cambridge: Cambridge University Press 71–93.
- Singh DP and Sarkar RK (2014) Distinction and characterization of salinity tolerant and sensitive rice cultivars as probed by the chlorophyll fluorescence characteristics and growth parameters. Functional Plant Biology 41(7):727–736.
- Smirnoff N (1993) The role of active oxygen in the response of plants to water deficit and desiccation. New Phytologist 125(1):27-58.
- Swain P, Raman A, Singh SP and Kumar A (2017) Breeding drought tolerant rice for shallow rainfed ecosystem of eastern India. Field Crops Research 209:168-178.
- Talwar HS, Prabhakar M. Elangovan, Aruna Kumari, SS Rao, JS Mishra and JV Patil (2011) Strategies to improve post flowering drought tolerance in rabi sorghum for predicted climate change scenario. Crop Improvement 37(2):93-99.
- Tian L, Tan L, Liu F, Cai H and Sun C (2011) Identification of quantitative trait loci associated with salt tolerance at seedling stage from Oryza rufipogon. Journal of Genetics and Genomics 38(12):593-601.
- Uga Y, Sugimoto K, Ogawa S, Rane J, Ishitani M, Hara N, Kitomi Y, Inukai Y, Ono K, Kanno N and Inoue H (2013) Control of root system architecture by DEEPER ROOTING 1 increases rice yield under drought conditions. Nature Genetics 45(9):1097-1102.
- Wang WB, Kim YH, Lee HS, Kim KY, Deng XP, Kwak SS (2009) Analysis of antioxidant enzyme activity during germination of alfalfa under salt and drought stresses. Plant Physiology and Biochemistry 47(7):570-577.



- Xu K, Xia X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC and Mackill DJ (2006) Sub1A is an ethylene response factor-like gene that confers submergence tolerance to rice. Nature 442(7103):705–708.
- Xu K, Xu X, Ronald PC and Mackill DJ (2000) A high-resolution linkage map of the vicinity of the rice submergence tolerance locus Sub1. Molecular and General Genetics 263(4):681-689.
- Yoshimoto M, Fukuoka M, Hasegawa T, Matsui T, Tian X, Vijayalakshmi C, Singh MP, Myint TT, Weerakoon WMW, Lafarge T and Lur HS (2012) MINCERnet: A global research alliance to support the fight against heat stress in rice. Journal of Agricultural Meteorology 68(2):149-157.
- Zeng L, Poss J, Wilson C, Draz AS, Gregorio G and Grieve C (2003) Evaluation of salt tolerance in rice genotypes by physiological characters. Euphytica 129(3):281-292.\*



## Innovative Extension Approaches for Increasing Income of Rice Farmers

SK Mishra, L Das, GAK Kumar, NC Rath, B Mondal, NN Jambhulkar, P Samal, BN Sadangi, SK Pradhan, S Saha, PC Rath, AK Mukherjee, RK Sahu, PK Guru, CV Singh, SM Prasad, S Bhagat, S Roy, R Bhagawati, K Saikia and SK Ghritlahre

#### SUMMARY

Social research on development and experimentation with new extension approaches and models for faster dissemination of latest technologies and their wider adoption has got more significance after Green Revolution during late 1960s in India. These approaches vary widely from top-down and supply-led to bottom-up, demandled and participatory, mostly due to diverse agro-climatic regions, socio-economic conditions and types of stakeholders. The ICAR-NRRI has been experimenting with such five innovative extension approaches relating to (i) rice varietal popularization, (ii) sustainable local seed system, (iii) rice value chain, (iv) gender sensitive extension and (v) rice-based climate smart model village approach. The rice varietal popularization strategy aims at shortening the time gap between the development of a new higher yielding superior variety and its wide adoption by farmers. This would also help in substitution of old mega varieties susceptible to various pests and diseases with superior and multiple stress tolerant varieties in view of changing climate scenario. The 4S4R model of local seed system has attempted to improve the local seed system of villages through formation of farmer producer organizations (FPOs)/farmer interest groups (FIGs) and capacity building of farmers in quality seed production of their locally demanded varieties, in right time and at lower cost. This self-sufficient sustainable seed system would help to address the various acknowledged demerits of the public seed system. The rice value chain model developed through an MOU of stakeholders from rice breeder to consumer would ensure rice growers to get fair price of their produce, while benefiting all stakeholders and satisfying the consumers. The gender sensitive extension approach in a rice-based farming system would help in proper utilization of latent capacities of farm women and grooming them to lead agrarian economy at par with their male counterparts. This approach would also help to change the traditional mindset of the male-dominated society and create a healthy societal climate boosting rural economic growth. The access to technologies and productive resources by farmwomen has increased remarkably in our study area. The rice-based climate smart model village approach through coordination and convergence of all development departments alongside the farming communities has helped to bring all stakeholders to a single platform for holistic planning of villages, execution of action plans, monitoring of interventions and immediate remedial measures. This approach would help to mobilize the farmers, farmwomen and rural youths, while addressing issues relating to livelihoods, youth unemployment, market linkage and changing climate.



## **1. INTRODUCTION**

Development of new approaches in agricultural extension in India and worldwide is a continuous process with its focus on increasing productivity and profitability. Since the Green Revolution during late 1960s, Indian agricultural extension has adopted decentralized, participatory and demand-driven approaches, in which accountability is geared toward the users (Kokate et al. 2009; Sulaiman and Hall 2008; Swanson 2009). While the call for demand-driven agricultural extension has existed for several decades now, new modes of reaching out to farmers could have significant impact in India, as they might better reflect the local information needs of farming communities. The diverse nature of the Indian subcontinent, with its wide variety of agro-climatic regions and broad range of socio-economic conditions in the rural population, calls for agricultural extension approaches that are context-specific and situation-specific. Extension organizations in general have been using a wide range of methods for reaching individuals, groups and the wider public in rural areas with new information/ knowledge. Approaches to extension also vary widely from top-down and supply-led to bottom-up, demand-led and participatory. Approaches also vary depending on the mandate of the organization or the programme. Advances in information and communication technologies (ICTs) have also provided new opportunities for extension to reach more farmers in a short amount of time (Sulaiman et al. 2011). Five such innovative extension approaches pertaining to (a) rice varietal popularization, (b) sustainable local seed system, (c) rice value chain, (d) gender sensitive extension and (e) rice-based climate smart model village approaches have been covered in this chapter. Efforts have been made through the chapter to address various issues and problems responsible for low income of farmers and share innovative ways, means and extension solutions to get rid of those problems. The new ideas could be suitably blended with existing extension models to hasten the extension service delivery in any developing nations. The authors are of strong opinion that the information on innovative extension approaches would be very useful to researchers, policy makers, academicians, development professionals, agro-processing industries, scholars and farmers at large.

## 2. CONSTRAINTS OF TECHNOLOGY TRANSFER AND ADOPTION

The varietal development effort of the ICAR-NRRI has got an impetus in recent past with an average of about six varieties per year (Table 1). But most of these varieties are neither in state seed chain nor adopted by farmers due to their unawareness or any other reason.

Period	No. of years	No. of variety developed	Average no. of variety developed per year
1946-2000	54	57	1.05
2001-2010	10	28	2.80
2011-2017	7	40	5.71
Total	71	125	1.76

#### Table 1. No. of rice varieties developed by ICAR-NRRI since its inception.



A critical review of past studies reveals that the major reasons for slow spread and adoption of new varieties by farmers pertain to (i) non-inclusion of recent varieties in state seed chains, (ii) non-receipt of breeder seed indents from the state agriculture departments, (iii) lack of sufficient quantity of quality seeds, (iv) lack of publicity and awareness among farmers and extension personnel, (v) insufficient minikit trials and demonstration programmes, (vi) lack of effort by central and state extension machineries, (vii) absence of suitable seed production and distribution policy etc. It is seen that a new rice variety takes about 6-8 years to be known or popular by the farmers. But as per the existing government policy, all the subsidies cease for any variety which is older than 10 years and those cannot be promoted through any scheme with exception in case to case basis. To overcome this problem, the institute has intensified its effort to fast spread and popularize newly released varieties in various states through front line demonstrations and other transfer of technologies methods with the active participation of various stakeholders.

As per the feedback information collected by this Institute from the rice growers of our country, unavailability of quality seeds in sufficient quantity and in right time is their most pressing problem. The formal system of seed production in India has been fulfilling the need of the farmers. However, the system faces a number of problems related to quality, quantity, timeliness, choice of variety, cost of seed production and distribution. The solution lies in developing suitable local seed systems. In contrast to formal seed sector, local seed system, if strengthened, can offer solutions to overcome the constraints of formal (government) seed supply system. Local seed system can produce seed according to local farmers' need, in right quantity, of right quality, with lower cost of production and supply and with timely delivery of seed to farmers. Therefore, this institute has developed a self-sustaining local seed system model, which needs to be validated before being replicated and adopted in a large scale. This will help in accelerating the varietal replacement rate (VRR) as well as seed replacement rate (SRR) in rice.

One of the major concerns of farmers is the absence of lack of proper marketing facilities in almost all the states. Farmers are deprived of getting their minimum support price (MSP) and are forced to sell at distressed price (less than 2/3<sup>rd</sup> of the MSP) to the middlemen. To ensure fair price of their produce and maximize rice farmers' income and net profit, the ICAR-NRRI has been working on developing and refining a multi stakeholders' rice value chain (RVC) approach since 2014-15. Among the various approaches to increase farm income and promote entrepreneurship, the prospect of rice value chain assumes importance in agriculture and allied sectors (Das 2017). The RVC, besides having the fundamental benefits, has some added prospective like (i) rice will continue to dominate the farm production for various socio-economic and cultural reasons in spite of poor financial gains and market glut, (ii) demand in the national and international market for quality rice is quite apparent, (iii) apart from farmers, other stake holders can join the chain leading to creation of additional employment and (iv) quality and specialty rice varieties developed by research institutes can spread quickly with less investment in extension.



Gender issues in rice farming have always been a much talked topic of discussion. Rice production is a labour-intensive activity, which involves both male and female members. In eastern India, farmwomen perform up to eighty per cent of the work in rice fields and are involved in almost all activities of rice farming. However, they are often marginalized in business relations and have minimal control over access to factors of production like land, inputs such as seed and fertilizer, credit and technology. So, the question remains how to empower farm women, when the available statistics speak volume of their poor condition worldwide. It is reported that unlike farmers, only five per cent of current agricultural extension efforts and resources are directed to farmwomen. Secondly, India is a country of continental size with a population of over 1270 million, out of which about one third live in urban areas. Ready-to-eat processed and packaged foods have become necessity and popular among this huge urban population in recent years. Therefore, the Indian food processing industries including rice is being termed as a 'sun-rise industries' and several efforts have been made in the last few years to give a big thrust to this sector. The food processing sector plays an important role in improving agricultural productivity, reducing postharvest losses, providing better nutrition, creating huge employment opportunities and in improving food availability for the domestic market. Looking at the inherent skills and expertise of Indian women in preparation of traditional rice based value added products, we need to harness this vast pool of knowledge and skill, and translate them into a commercial venture providing livelihood security, food and nutritional security, and contributing to national economy. Therefore, we should focus on some of these issues and strategies for commercialization of such rice based processed value added products (VAPs) in an entrepreneurial mode and linking them to the food value chain the country.

Several agencies are working for developing rural areas and village people. The development takes place on the basis of quantum of efforts of agencies in their respective areas of jurisdiction. The development is not always uniform. Piecemeal approach, sporadic efforts and casual attitudes of development agencies often lead to skewed growth and development. The visibility of efforts tends to disappear slowly or fast depending upon the magnitude and quality of work, once the change agents/ development professionals withdraw their involvement. When different agencies work independently in different directions in meeting the aspirations and expectations of the village people, the focus of attaining sustainable rural development is lost. This results in uneven development. Hence, this calls for an integrated extension approach involving different stakeholders in ensuring holistic development of villages. This can also lead to development of model villages where progressive agriculture and empowered village society would be witnessed. Developing a rice-based climate smart model village through convergence of all stakeholders namely, researchers, development personnel, farmers, farm women, youths, farmers' organizations and NGOs etc is another innovative extension approach for inclusive development of all available local resources in an integrated manner.



## 3. STATUS OF RESEARCH

Regarding adoption of rice varieties in Bangladesh, Hossain (2012) reported that the number of varieties grown in different seasons were 1091 (Aman/Kharif-535, Boro/Summer-261 and Aus/Autumn-295). However, only a few varieties covered a large proportion of area. The major varieties according to area coverage in Bangladesh were BRRI Dhan 29 (37%) and BRRI Dhan 28 (23%) in dry season, while BR 11 (27%) and Swarna (12%) in wet season. Similarly, the survey report also encompasses findings from three major rice growing eastern Indian states namely, West Bengal, Odisha and Jharkhand. The survey identified 226 rice varieties in West Bengal, a large proportion of which were traditional varieties mostly grown in the aman season. The most popular variety in the aman season was found to be Swarna (45% of the rice area), whereas in the boro season it was IR 36 (27% area). The main source of seed for aman varieties was farmers' own seed, whereas, in the case of improved boro varieties, it was seed traders. In case of Odisha, the number of varieties identified in the wet season was 723, most of which were traditional varieties. On the other hand, the number of varieties in the summer season was 29, all of which were improved varieties grown under irrigated conditions. Variety Swarna (29.3%) was the most popular variety in the wet season, whereas in summer it was Lalat (47.0%). In Jharkhand, altogether, 145 varieties were identified and the highest number was for medium land (71), followed by lowland (55) and highland (19). In the highland, traditional variety Gora Dhan was found to be the most popular, while in the medium land and lowland, improved varieties namely IR 36 and Swarna respectively, were the most popular varieties.

Regarding primary traits for farmers' varietal preferences, he reported that farmers sought high grain yield from limited farm size as the most important trait in a new variety. The responses of the farmers from Bangladesh, West Bengal, Odisha and Jharkhand with respect to this higher grain yield trait were 96%, 100%, 100% and 73% respectively. Farmers also looked for secondary traits like grain quality for a premium price in the market, shorter maturity duration, lodging resistance, and higher milling recovery.

The survey on adoption and diffusion of new varieties in Bangladesh, West Bengal, Orissa and Jharkhand revealed that (i) If varietal performance is substantially better than that of existing varieties, large farmers adopt and small and medium farmers follow, otherwise, the variety is eliminated, (ii) Availability of seed of improved varieties is a major constraint for fast-tracking diffusion (70% to 80% of the seeds are from farmers' own harvest or are exchanged with or purchased from neighbors), (iii) Once farmers in a village are convinced of the superiority of a new variety, it takes 3 to 5 years to reach areas suitable for the variety, (iv) However, it may take a longer time to reach a substantial portion of area because of information lag (extension system is not highly effective, radio/television is a minor source of information, input dealers are not targeted as information bearers), and (v) Once a variety is established, it is difficult to dislodge it, unless new improved varieties have traits that are substantially superior.



For realizing optimum productivity of any crop in any production environment, the choice of an appropriate variety is extremely essential (Lal 2010). He added that the variety to be selected for cultivation must be adapted to the specific agro-ecologies/ production environments. Improper choice of the variety would result in low productivity, even when adequate quantities of inputs are applied. It is equally important to use the latest recommended varieties, since all varieties tend to lose disease resistance on account of evolution of pathotypes/ biotypes of the disease.

For promoting newly released varieties, ICAR-NRRI has been producing breeder seeds of rice as per seasonal indents received from various states through the Department of Agriculture & Cooperation, Government of India and various other organizations. In addition, truthfully labeled (TL) seeds are produced in the research farm as well as in farmers' field in a 'Farmer Participatory and Buy-back mode' for direct sale to farmers through its own sale counter. In addition, limited varietal demonstrations are conducted in selected clusters in the Odisha state. As part of the varietal development programme, all India coordinated research project (AICRP) trials are conducted in various states to test the efficacy and adaptability of the varieties. Apart from these, minikit trials are conducted through various research initiatives like institutional research projects, externally aided projects, and various programmes like, Tribal Sub-Plan (TSP), Mera Gaon Mera Gaurav (MGMG), Farmer FIRST, BGREI, NFSM, NICRA, KVKs etc to demonstrate and promote new varieties. The institute also organizes and participates in national and international exhibitions showcasing its latest varieties and rice production technologies. All trainee farmers and important stakeholders coming from various states are also provided with seed minikits for their respective local demonstrations and spread.

Paroda (2010) opined that for achieving desirable levels of seed replacement rate (SRR), adequate seed needs to be produced first. Seed production programme should be organized in each state under a comprehensive and integrated state seed plan appropriate to region specific requirements. States should ensure production, multiplication and replacement of seed and varieties to increase seed multiplication ratio (SMR), seed replacement ratio (SRR) and variety replacement ratio (VRR) progressively, particularly in respect of regionally important crops/varieties. While delivering a special lecture during Indian Seed Congress 2013, Paroda (2013) mentioned that promotion of hybrids/ HYVs in major field crops should be a high priority to bridge the productivity gap and increase production. In this context, the private sector has to play a major role. Immediate action is warranted for phasing out of all old and obsolete varieties through de-notification and promoting only the best varieties and hybrids suitable for specific regions, irrespective of whether they are from public or private sector.

Sharma et al. (2013) stated that the present supply chain structure of rice in India works on the traditional framework, which involves many intermediaries at supply and distribution fronts. The current supply chain structure of rice in India is somewhere lacking in efficiency and needs reforms. The traditional supply chain structure faces the problems of inventory management, where either there is the

485



overstocking which results in obsolescence and increased supply chain costs, or the stock outs of the demanded varieties resulting in lost sales. Supply chain of rice in India is also facing the supply chain problems related to procurement, distribution, intermediaries collaboration, and logistics system which needs to be redesigned. In spite of being the second largest producer in the world and a big consumer of rice, which holds a significant presence in the global agri-food market, India fails to contribute to the global food business to the level it deserves.

While analyzing value chain of rice and wheat in Uttar Pradesh state of India, McCarthy (2008) viewed that high local, global and regional demand for rice and wheat could greatly benefit smallholder farmers of both of these staple crops in the rural areas of India. Small landholders, the major engines of production in India, can take advantage of these growth trends to meet this demand and increase their incomes. Taking advantage of these parallel trends will require farmers to increase production, reduce post-harvest losses and market their crops in new ways. Amendments to the restrictive state marketing channel (through mandis, or wholesale markets) are beginning to allow farmers to access more profitable channels for their produce. The mandi system does not reward farmers for higher-quality produce as alternative market channels would, such as direct supply to supermarkets by establishing value chain. The major actors in the rice and wheat value chains are input suppliers (including manufacturers, wholesalers and retailers); producers; a large number of intermediaries (including collectors, traders, commission agents, and brokers); wholesalers; processors (including rice and flour millers); and retailers.

There is a growing body of evidence that promoting women's rights over land and natural resources are keys to enhancing women's livelihood security and promoting women's empowerment. Land ownership is likely to have positive effects on agricultural productivity, food security and children's education (Agarwal 2003). Moreover, technology research and innovations are rarely focused on women's specific needs and roles. As a result, farm women generally lack access to improved technologies for use in farming activities, and the large majority of them still rely on traditional, labour-intensive, drudgery-prone and time-consuming technologies. In many countries, the menu of options available to farmers has become more diversified. For instance, in Ethiopia the creation of a 'women's development package' indicates that agricultural officials are trying to improve their services to women (Tewodaj et al. 2009).

The ICAR-NRRI, which has been mandated to increase production and productivity of rice-based farming systems in India, took up a project on 'Gender Mainstreaming in Rice' during 12<sup>th</sup> plan period (2012-2017). A socially acceptable, practicable and replicable approach was conceptualized based on the hypothesis that, 'if the inherent capacity of the farm women are explored and built up through sensitization, gender gap identification, awareness, training, exposure to technologies, access to family resources, group mobilization alongside the male counterparts of the society and necessary organizational support, then all round development of the farm women and household agricultural production and productivity can be achieved'.



Under this approach, intense gender sensitization was a pre-requisite, which followed capacity building programmes of women rice growers. Both men and women get equal opportunity to exchange their experiences and feelings to provide community support to women rice growers in many critical areas of gender gap. Finally the approach ends in leaving the women in the family and evaluating the overall household improvement in rice production and productivity in particular and gender issues in general. The major activities, outputs, outcomes and experiences have been discussed below under the approaches section.

## 4. KNOWLEDGE GAPS

Results of on-station, off-station and on-farm demonstrations, minikit trials and farmers feedback on the institute developed varietal performance and their superiority over local popular checks have been very encouraging over the years. Despite all above efforts by the institute, varieties are spreading slowly and not reaching a wider population as desired. One of the major shortcomings of the institute is production of limited quantity of seed due to availability of limited area of about 30-40 hectares land for seed production. On the contrary, state agricultural universities have been wellplaced due to their huge network of regional research stations within respective states with vast area under seed production plan. Apart from superiority of varieties, availability of large amount of quality seeds (foundation and certified seeds) is equally essential to fulfill the seed requirements of state machineries to promote through seed chains. Because of this reason, ANGRAU model of rice varietal diffusion and popularization through distribution of sufficient minikits during initial 2-3 years of their development has been a very successful model, as was shared by two of its former Breeders-cum-Vice Chancellors namely, Prof. P. Raghava Reddy and Prof. Padma Raju during the national level Brainstorming Workshop on "Rice Varietal Diffusion: Estimation, Problems and Prospects" organized by MANAGE at Hyderabad during 19-20 May, 2017, citing examples of mega varieties like Swarna (MTU-7029), MTU-1010, MTU-1001 and Samba Mahsuri (BPT-5204).

Another grey area is, farmers are not getting quality seeds in time at their door step, even though rice seed market is growing faster in recent years. Rice seed production and marketing is a very good enterprise in itself. Still, many Indian states are facing very acute shortage of rice seeds with very less VRR and SRR. Rice seed production technology and marketing can easily be promoted and adaptable, but requires proper technical backstopping and active participation of farmers' organizations. There is hardly any research or extension or organizational efforts to make rice farmers self-sufficient with quality seeds at local level. Similarly, this institute has developed some high protein, aromatic, export quality long slender and superfine grain varieties, which can fetch higher remunerative market prices, benefitting the rice growers. Developing and refining a multi-stake holder's rice value chain (RVC), involving all players starting from the rice developer to the consumer, can be a unique and innovative proposition for this.



Indian farmwomen play a more significant role in rice sector contributing to substantial increase in family income. They are traditionally skilled to prepare thousands of value-added food products, so also rice based products. Encasing their skills and involving in rice based value added enterprises and linking them to the market can be a successful research endeavour. But, no such visible steps have been taken so far at any level.

### 5. RESEARCH AND DEVELOPMENT NEEDS

In this section, various processes involved and experiences learnt in all the five innovative extension approaches of NRRI, Cuttack have been discussed.

#### 5.1. Approach-I: Rice varietal popularization strategy

The Institute is working on developing mechanisms to shorten the period between varietal development and varietal spread leading to wide adoption, which can be simplified in following concurrent activities. First of all, we need to identify existing popular varieties and farmers' preference in selected states for testing new and comparable improved varieties through collection of primary as well as secondary data from targeted areas. Accordingly, taking all criteria like ecology, duration and farmers' preference into consideration, a 'Varietal Matrix' can be prepared for all 'popular but lower yielding varieties' vis-a-vis 'new, superior and higher yielding varieties' for replacement with better alternatives. Ecology wise district clusters should be selected (may be, any one revenue block close to the district headquarters and adjacent to a primary village road) and selection of about 15-20 innovative farmers per cluster in consultation with state agriculture department officials and other stakeholders. Varietal demonstration should be conducted by providing only 5-10 kg seed minikits as critical inputs without altering farmers' own crop management practices. Planning for demonstration should be done in such a way that all the districts may be covered in a maximum of 2-3 years in rotation.

These small scale on-farm demonstration may be done for participatory varietal evaluation in consultation and collaboration with all stakeholders like, state departments of agriculture (SDAs), state seed corporations (SSCs), state seed certification agencies (SSCAs), farm science centres/ krishi vigyan kendras (KVKs), state agricultural universities (SAUs), regional research institutes, farmer interest groups (FIGs), private seed companies and dealers, non-governmental organizations (NGOs) working in agriculture sector, media representatives and both demonstrating and non-demonstrating farmers. Big size and clearly visible road side field boards should be placed on the demonstration sites with details of varietal characteristics in local language.

Capacity building programmes have to be conducted for various stakeholders through training programmes, package demonstration, technical backstopping through field visits/telephonic advisory/creating mobile social groups and conducting field days at various stages of crop growth, especially in pre-harvesting stage associated with crop cutting experiments, with the principle of 'Seeing is Believing', involving all the stakeholders including non-demonstrating farmers to showcase the



superiority of the new varieties. Participating farmers should be encouraged to share their experiences to motivate fellow stakeholders. Best performing new varieties should be upscaled through creating demand for breeder seed indents from next year onwards and promotion of local seed production by government and private agencies for making timely seed availability to farmers, and creating an institutional mechanism for planning and production of adequate quantity of seed for minikit distribution. A nodal officer along with a team of experts may be identified for continuous monitoring at the institute as well as state levels.

Rigorous awareness campaign is required through electronic media, print media, ICT tools like mobile apps/ social groups and distribution of extension leaflets in local languages. Preparing success stories, recording of farmers' reactions and overall processes documentation are required for publicity and distribution. State level workshops in the initial years must be conducted involving policy makers and senior state development departments, officials to create awareness and convince key players about the superiority of newly developed varieties. The non-conventional channels (like seed companies, rice millers, traders and food processing industries) have to be explored for spread of remunerative varieties. For fast spread of varieties, advisory should be issued to participating farmers for not consuming the produced grains of demonstrated plots during initial years, rather encouraging 'farmers-to-farmers' horizontal spread of seeds either through sale or on barter basis for rapid spread. As part of the process, a good document should be prepared encompassing the workshop proceedings, action points, demonstration details, crop cutting data vis-à-vis comparative data on local varietal performance, feedback from farmers and other stakeholders. The document should be widely circulated among important state and central level officials & policy makers and action points should be followed up accordingly.

## 5.2. Approach-II: Devising a self-sufficient sustainable seed system for rice (4S4R)

The NRRI developed Self-sufficient Sustainable Seed System for Rice (4S4R) model was conceptualized and developed in 2014-15 and since then it is being implemented, tested and refined in Mahanga block of Cuttack, Odisha. However, the model itself is general in nature harnessing the advantages of advancements in information technology (IT) sector at various stages, like planning, execution, monitoring, capacity building, support and marketing. The model tries to combine best of the technologies and practices available with research institutions, universities and IT institutions. Existing seed system was improved and supported by various innovative interventions as follows.

- Facilitated farmers' access to seed through (a) Awareness, (b) Training, and (c) Capacity building;
- Introduced appropriate agricultural technologies in (a) Crop production, (b) Integrated pest and disease management, (c) Introduction of improved varieties, and (d) Seed health and storage management;
- Improved disorganized local seed system through (a) Improved organization by starting Farmer Producer Organisation (FPO), (b) Registering FPO under Company



Act 2013 (Old 1956) as Mahanga Agro Producers 4S4R Pvt. Ltd. on 30 April, 2017, and (c) Providing support for establishment and sustainability; and

Provided IT based solutions for (a) Expert system of seed production – 'Paddy SeedXpert' was developed which is available at Google Play Store, (b) Used remote sensing for identification of appropriate location for seed production, (c) Used remote sensing and GIS maps to determine the seed requirement of the area, (d) Linked financial institutions/KVKs of the districts to the Farmer Producer Organisation (FPO), and (e) GIS mapping of seed availability and marketing.

This model has FPO in the centre of the activity at a block level (Fig. 1). The FPO consists of i) seed producing farmers' group, ii) seed processing enterprise and iii) seed selling and marketing enterprise mainly catering the quality seed requirement of

the block at local level. The seed producing farmers' group produces foundation/certified seeds as per local demand from the breeder seed supplied by NRRI. Paddy seed processing and packaging machineries besides seed storage godowns have been provided through Rastriva Krishi Vikas Yojana (RKVY). The sale outlet is part of the processing unit. These two units have been developed using

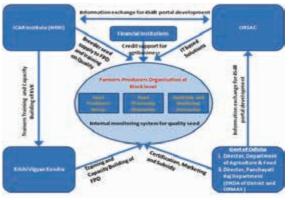


Fig. 1. Schematic representation of the 4S4R Model of NRRI.

entrepreneurship development approach. Specialized training programmes are imparted in the area of FPO management and paddy seed production which are followed by required support to establish processing and marketing unit(s).

The pivotal role in this model is being played by NRRI, being the specialized institute for technology development in rice. The institute performs specialized roles like, (i) supplying breeder seeds of locally preferred rice varieties. (Besides NRRI, OUAT, Bhubaneswar also supplies breeder seeds to FPO as per the requirement of the farmers), (ii) providing production and post-production technologies for quality seed production, (iii) imparting training to KVK personnel involved in the project activities, (iv) providing inputs for expert system development for seed production in Odisha to IT Institution, (v) supporting in developing GIS-based tools for site (land) selection for seed production, (vi) organising workshops at planning stage, portal development stage and for capacity building to efficiently implement the project, and (vii) coordinating various stakeholders in achieving the objectives of the project.

So far as the economic benefits of the model is concerned, the 'Mahanga Agro Producer 4S4R Pvt Ltd', registered as part of the research initiative by NRRI with participation of over hundred farmers, initially required nearly Rs. 31.94 lakhs as cost



of the project, but in the second year itself, the Break Even Point (BEP) was achieved with Rs. 87.0 lakhs sales realisation. The major challenges faced in implementing this project are coordination among different stakeholders besides setting up FPOs at block level and marketing of the paddy seed.

## 5.3. Approach-III: Developing a replicable rice value chain benefiting all stakeholders

The main objective of the NRRI developed rice value chain (RVC) was to promote large scale cultivation of high quality and specialty rice varieties of this institute in contiguous patches, and to undertake it's processing and trading, so that the consumers have access to premium quality rice and all the parties involved in the value chain are benefitted. During the planning phase of the RVC in 2014-15, several brainstorming sessions, consultations and focused group discussions were held

with all stakeholders including state agriculture department to decide the objectives, stakeholders, activities, links, responsibilities of the partners and benefits sharing. Finally, a chain emerged in public-privatepartnership (PPP) mode with the involvement for five parties including ICAR-NRRI, Cuttack (Fig. 2). In consultation with all stakeholders, a long slender grain aromatic inbred rice variety 'Geetanjali' developed by NRRI was selected for the rice value chain during the initial year. The responsibilities and benefits for each party were decided and agreed upon (Fig. 3) through memorandum of understanding (MOU), in brief as follows.

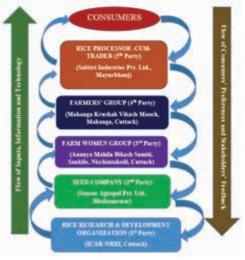


Fig. 2. Schematic representation of the RVC Model of NRRI.

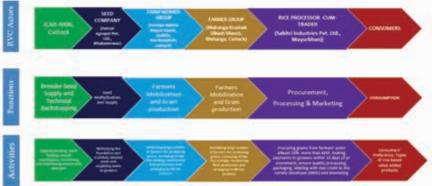


Fig. 3. Functions and Activities of RVC Actors.

Innovative Extension Approaches for Increasing Income of Rice Farmers



- 1<sup>st</sup> Party (ICAR-NRRI, Cuttack): for supplying breeder seeds of 'Geetanjali', technical backstopping, capacity building, hand-holding, overall coordination, monitoring, and maintaining season-wise database.
- 2<sup>nd</sup> Party (Sansar Agropol Pvt. Ltd., Bhubaneswar): A seed company for multiplying the foundation or truthfully labeled seeds and supplying seeds to farmers groups/ rice growers at desired destination in time.
- 3<sup>rd</sup> Party (Ananya Mahila Bikash Samiti, Sankilo, Nischintakoili, Cuttack): A farm women group for mobilizing large number of farmers and producing large quantity of grains.
- 4<sup>th</sup> Party (Mahanga Krushak Vikas Manch, Mahanga, Cuttack): A farmers group for mobilizing large number of farmers and producing grains. Apart from production, these farmers groups were also involved to undertake survey of the rice ecology, motivate farmers of various districts of the state to participate in the chain, monitor the production and arrange lifting of production by the rice processor-cum-trader.
- 5<sup>th</sup> Party (Sabitri Industries Pvt. Ltd., Mayurbhanj): Rice processor-cum-trader for procuring grains from farmers' point immediately after the harvest season ends, at a price of at least 20 percent above the minimum support price (MSP) fixed by the Govt. of India, making payments to growers within ten days of procurement, ensuring quality processing, packaging, labeling with due credit to the variety developer (NRRI) and marketing.

Intensive efforts were made for field monitoring, technical backstopping and capacity building through farmers training, distribution of extension literature in local languages and mobile advisory services through a monitoring committee comprising of multi-disciplinary scientists and state line department officials. Workshops at various stages of the cropping period (pre-kharif, pre-rabi and post-harvest etc) are conducted for deciding varieties, finalizing seed and grain production plan, number of farmers to be involved, recording feedback analysis, sharing experience, and resolving issues with the participation of all stakeholders. As part of our initial effort during rabi 2015-16, grain of Geetanjali variety was produced in three clusters totaling 166 acres of Khurda and Cuttack districts involving 82 farmers. The average yield of the crop was recorded at 4.0-4.5 t/ha. After keeping for seed and household consumption, 202 tons of paddy grains were sold by the participating farmers to the rice processor/ miller (5th party) at the rate of Rs. 17,400/- per ton (i.e., 20% above MSP) amounting to a total of Rs. 35.15 lakhs. Similarly, during kharif 2016 and rabi 2016-17, 136 tons of paddy grains were procured by the processor. The economic analysis shows that the participating farmers got an overall net income advantage of about 8-10 per cent over the non-participating farmers.

#### 5.4. Approach-IV: Gender sensitive extension approach in rice farming

The gender sensitive extension approach in rice farming was designed and tested in Sankilo village of Cuttack district with the involvement of over fifty participating



farm women during 2012-17 and is being carried forward in an entrepreneurial mode since then. The village was selected after making due consultations with the households and finding the social climate relatively better in gender sensitiveness. Preliminary meetings, gender sensitization programmes, gender gap analysis and PRA studies were undertaken by involving both male and female key informants separately to identify major gender issues in rice farming.

The major gender issues in rice farming identified included women-friendly technologies, access to resources & information, labour sharing, benefit sharing, capacity building, group mobilization, decision-making pattern, societal gender mindset, constraints in farming, linkage with financial & marketing institutions etc. Accordingly, suitable technological and institutional interventions were provided and evaluated. The male heads of the families/ the legal owners of lands were sensitized and motivated through personal contacts and close interactions to allocate about half an acre rice growing land to all the participating family farm women to take up crop demonstrations as per the advice of NRRI scientists and take independent decisions on crop management. A women development group in the name of 'Ánanya Mahila Bikash Samiti' was formed and registered after mobilizing all fifty farm women for deriving maximum institutional benefits and for group sustainability. Intensive awareness camps were organized and trainings imparted for desirable changes in their skill, knowledge and behaviour with regard to the objective of the programme, rice production technologies, market support and possible outcome of the project.

Demonstrations on rice production and crop management practices on popular and suitable rice varieties based on women's preference and market demand were conducted in the allotted half acre land by each farmwoman during kharif seasons. Apart from rice, during rabi seasons, technological interventions on cultivation of high value vegetable crops, pulse crops and preparation of value added food products were also given. Seeds and planting materials were provided free of cost as critical inputs during initial years only. Improved rice production technologies like growing of mat type nursery, seed treatment, line transplanting, use of rice transplanters, balanced dose of fertilizer application and need based pesticides application were provided along with technical backstopping in women's perspectives. Similarly, for harvesting and post-harvesting management, training-cum-demonstrations on drudgery-reducing and women-friendly machines and technologies like NRRI riceparboiling unit and NRRI rice-husk combustor; and demonstration on paddy-straw mushroom cultivation was also conducted for additional revenue generation and family nutrition from rice by-products.

Looking at their acquisition of enough technical competencies and managerial abilities, the group was made as a signatory to the NRRI developed rice value chain for ensuring greater economic benefits of the participating women members. Reactions of the farm women were recorded at regular intervals to assess the effects of interventions and modify accordingly, if called for. The major impacts of the project in terms of outputs and outcomes as found out through concurrent and end-term evaluations are briefly outlined below.

493



- a) Change in attitude towards gender mainstreaming: Significant change in attitude towards gender mainstreaming was established. The male members of the family as well as in the village are now giving more importance and recognition to the farm women in farm, family and community matters. More so, they were happy and motivated to see and watch the success stories of their village in print and electronic media. They are now allowing female members to attend agricultural meetings and programmes outside.
- b) Mindset of male members of family/society: Findings indicate that there was major change in the mindset of male members of family/society towards womenmanaged rice farming (90%). All the farm women were feeling recognized by other members of the family as well as village due to their increased capacity in farm and home management activities and leadership in organizing group and social activities.
- c) Competency of farm women: As opined by the farm women, remarkable changes in behavior of women rice growers were found with regard to agricultural knowledge (100%), technical skill (93.33%), decision-making capacity (86.67%) and undertaking group activities (76.67%). Improvement in skills in nursery raising, handling farm implements, and disease and pest management were also observed.
- d) Women friendly production technologies: Technologies with regards to raising of mat-type seedlings for mechanical transplanting, seed treatment, mechanical line transplanting and use of small farm equipments were found drudgery reducing. Among the women-friendly farm machineries demonstrated, rice husk combustor, finger weeder and 4-row manual drum seeder were perceived as more appropriate by 85.71%, 70.37% and 57.14% farm women respectively. Paddy straw mushroom cultivation as an income generating activity by converting rice byproduct (straw) was also rated as more appropriate by 88.46 per cent farmwomen.
- e) Perception about demonstrated technologies: All the participating farmwomen adopted scientific production practices based on their socio-economic feasibilities. The analysis of the data shows that majority of the respondents had positive perceptions with regards to comparative advantage of recommended/demonstrated rice varieties over earlier grown varieties in terms of yield, resistance to pest/ diseases, tolerance to weeds/drought, labour saving, profitability and marketability.
- f) Access to productive resources: Significantly increased access of women to farm inputs was observed through the approach, as evident from the expansion of allotted half an acre land to over one acre in many families by end of 2-3 years. This expansion of area under the control of farmwomen signifies more trust and confidence on women farmers by their male counterparts and a positive impact of the project. Similarly, accessibility to family land (100.0%), seeds (100.0%), fertilizers (100.0%), and money (45.45%) were found.
- g) Entrepreneurial opportunities: Since, the farm women had their expertise in preparing traditional value added food products (VAPs), they were encouraged and supported to convert the traditional value added rice products into demand driven marketable products through improved food technology process. A book

on 'Traditional Rice Foods - The Rich Heritage of India' was also brought out containing the processes of making over hundred traditional rice-based value added products, primarily collected from the women group members.

- h) Partnering in RVC: By working in groups, the women realized their inherent capacity, developed friendly atmosphere and learnt the importance of working in groups in the society. Accordingly the registered group also took up entrepreneurial activities as a signatory to the NRRI rice value chain and in turn potential women entrepreneurs were recognized.
- i) Outstanding public recognition: Among others, one of the successful farm women Smt. Rukmini Nayak of the group was conferred with 'Best Innovative Farmer Award' during 'Krishi Unnati Mela-2017' at IARI, New Delhi and with 'Best Farmer Award' during 'Akshay Tritiya & Farmers Fair-2016' at NRRI, Cuttack apart from several other awards and recognitions, and received these awards from Hon'ble Union Minister of Agriculture & Farmers Welfare, Govt. of India.

# 5.5. Approach-V: Developing a rice-based climate smart model village through convergence

The National Rice Research Institute took up an initiative to demonstrate and develop an extension approach for 'development of a rice-based climate smart model village' with a broad objective of achieving all round development of Indian villages in convergence mode with emphasis on sustainability and equity during 2012-17. It was conceptualized as a holistic developmental model in a convergence mode involving various stakeholders like NRRI scientists, line departments, development departments, farmers associations, farmers, farmwomen, rural youths etc for achieving capacity building and overall development of farming communities through agriculture and allied activities.

The development of rice-based climate smart model village, evaluation of interventions and recommendations were taken up in a rainfed cluster, namely Gurujang-Guali of Tangi-Choudwar block of Cuttack district in Odisha. The cluster was selected because most of the people were socio-economically disadvantaged, the population comprised of mixed castes and livelihood, mainly dependent on rainfed rice-based farming options and facing many adversaries of climate change. There were about 100 farm families in the cluster with a total population of about 800. Based on bench mark survey and PRA studies, suitable technological and institutional interventions were provided and evaluated. Participatory technology demonstrations on rice, vegetables, animal husbandry, fisheries, group vegetable farming by women and allied activities were conducted. Capacity building of beneficiary farmers, farmwomen and youth was done through training, farmers-resource persons interactions, exposure visits and continuous technical backstopping. Village level stakeholders' meetings and workshops were organized by involving officers of the stakeholders departments (agriculture, horticulture, animal husbandry, fisheries, soil conservation, irrigation, panchayatiraj, cooperative etc), farmers and farm women of the cluster and scientists of the institute for developing a holistic approach and



mechanism of convergence among the departments. The priority needs of the cluster were identified as rain water harvesting under watershed and minor irrigation programme, control of wild buffaloes and stray cattle during rabi season and management of weeds in rice and vegetable crops.

Seasonal and annual action plans developed for the purpose were monitored from time to time for their effective implementation in a participatory and convergence mode. The high yielding rice varieties namely Sahabhagidhan, Swarna Sub-1, Pooja, Ketekijoha, Varshadhan, CR Dhan 304, CR Dhan 202 and Naveen were most liked due to their various motivational traits. Climate resilient varieties namely, Sahabhagidhan (drought), Swarna Sub-1 (submergence), Varshadhan (deepwater & waterlogged), CR Dhan 202 (aerobic) etc. were introduced in the cluster and proved high adaptability and acceptance by the farmers. Prior to project interventions, they were cultivating HYVs of rice in about only fifteen per cent of rice growing area, which increased to about ninety percent at the end of five years. Farmers were still growing some local varieties due to their special grain and cooking quality.

The influence and benefits of group vegetable farming by women were very well noticeable and might help formation of more women groups for vegetable farming. The performance and impact were assessed which revealed that a very high percentage (94%) of farmers of model village had well derived various socio-economic benefits from the rice-pulse-horticulture-poultry-pond based production technologies propagated by the institute. The farming habit has changed. The fallow back yard lands of households have been covered under kitchen gardening or mushroom units or poultry units and the drought prone lands have been covered with drought resistant rice varieties or pulses. Apart from improvement in farming and socio-economic conditions of the villagers, village sanitation has also improved with the support from the block development department through construction of village pucca roads and individual household safety toilets. A study conducted in the cluster to assess the direction of changes revealed that significant positive changes had taken place with respect to attitude towards hybrid rice, knowledge on high yielding varieties, knowledge on rice cultivation, soil nutrient management, pest control and farm mechanization. Youths have been motivated to assist parents in farming activities or have taken up some kind of independent income-generating activities.

### 6. WAY FORWARD

As part of the institutional effort, cluster demonstrations were conducted during kharif 2017 involving 60 farmers in Jharkhand (with CR Dhan 202 and CR Dhan 305); 24 farmers in West Bengal (with CR Dhan 201, CR Dhan 203 and CR Dhan 304) and about 200 farmers in Odisha with 20 new rice varieties released for the state. The crop cutting results of almost all the demonstrated varieties showed a grain yield advantages of about 10-20% over all the existing popular varieties. A demand for seeds of these varieties has been created among the farmers. There is need to upscale these activities in convergence with other stakeholders in years to come.

Information technology and FPO are effectively integrated in 4S4R model which makes seed available at local level to ALL farmers according to their NEED, in right QUANTITY, of right QUALITY, at lower COST and TIMELY delivery, which the present formal seed system has FAILED to deliver. This project activity enriched the subject on IT/ICT application in agriculture in general and dissemination of technology in particular. This has involved expert systems, GIS, MIS, web-based applications and mobile based methods for information dissemination. Another aspect that activity this project contributed, is development of approach to form FPO as an extension method for planned and organized transfer of technology and marketing.

The rice value chain approach has to be validated by widening the stakeholders' base, involving more number of rice millers, processors, traders and farmers organizations to generate competitiveness. Apart from long grain aromatic varieties, high value non-aromatic and nutritionally enriched high protein, high zinc rice varieties can be put into the chain. Nutritional rice varieties enriched with protein, iron and zinc can be promoted through government welfare schemes like mid-day meal for school children and Antyodoya AnnaYojana. Studies on marketing through e-national agricultural marketing (e-NAM) portal could be explored.

Access to productive resources is critical for enhancing women's economic choices. Since, formal credit institutions rarely lend to this weaker sex, special institutional arrangements has become necessary to extend credit to those who have no collateral to finance their enterprise. In order to have access to credit, social, institutional and government support is required. More than half of the farm labour is contributed by farm women. Moreover, as evident from several literatures, they have also proven their competencies over time and again to manage farm and home efficiently and effectively at par with the male members of the society, provided they were supported socially, economically, morally, technologically and institutionally. There is a need to identify their hidden capacities and entrepreneurial abilities and link them to the market. If they can be made technologically competent and socio-economically empowered, they could be the efficient drivers in achieving accelerated agricultural growth and development of the country in general and in boosting family income in particular. Organizing women into groups has been proved to be a good intervention. It can transform women from the status of 'beneficiaries' into 'clients', who are in a long-term can have a reciprocal relationship with the institutions meant to serve them

The model village approach has successfully integrated all stakeholders in the development of rural India in a participatory and convergence mode. In addition to addressing the issue of socio-economic and agricultural development, climate issues have been suitably addressed. The model needs to be validated and refined in other parts of the country and should be replicated in bringing prosperity to the society.



#### References

- Agarwal B (2003) Gender and Land Rights Revisited: Exploring New Prospects via the State, Family and Market. Journal of Agrarian Change. 1(1& 2). London: Blackwell Publishing LTD.
- Das Lipi, Sharma SG, Samal P, Patnaik SSC, Sahu RK, Rath PC, Mishra SK, Panda BB and Mukherjee AK (2017) Success Story on 'Rice value chain in PPP mode for increasing farm income and entrepreneurship', 1-4. ICAR-NRRI, Cuttack.
- Hossain M (2012) Rice varietal diversity, milling, and cooking in Bangladesh and eastern India: a synthesis. In: 'Adoption and diffusion of modern rice varieties in Bangladesh and eastern India. (Ed. M. Hossain, W.M.H. Jaim, T.R. Paris, and B. Hardy), International Rice Research Institute (IRRI), Philippines.
- Kokate KD, Kharde PB, Patil SS and Deshmukh BA (2009) Farmers-led extension: Experiences and road ahead. Indian Research Journal of Extension Education 9(2):18–21.
- Lal S (2010) Guidelines for selection of improved varieties/hybrids of rice, wheat and pulses for NFSM states. Manual. DAC&FW, Ministry of Agriculture & Farmers Welfare, Govt. of India, New Delhi.
- McCarthy S, Singh DD, and Schiff H (2008) Value Chain Analysis of Wheat and Rice in Uttar Pradesh, India, ACDI/VOCA for World Vision, Lucknow, India.
- Paroda RS (2010) Revitalizing Indian Seed Sector for Accelerated Agricultural Growth. Foundation Day Lecture delivered in the National Seed Association of India (NSAI) on 30 October, 2010.
- Paroda RS (2013) Indian Seed Sector: The Way Forward. Special lecture delivered in the Indian Seed Congress-2013, organized by NSAI on 8 February, 2013.
- Sharma V, Giri S and Rai SS (2013) Supply Chain Management of Rice in India: A Rice Processing Company's Perspective, International Journal of Managing Value and Supply Chains (IJMVSC), 4(1), March 2013.
- Sulaiman VR, Kalaivani NJ, Nimisha Mittal and Ramasundaram P (2011) ICTs and Empowerment of Indian Rural Women - What can we learn from on-going initiatives? CRISP Working Paper 2011-001.
- Sulaiman RV and Andy Hall (2008) The fallacy of universal solutions in extension: Is ATMA the new T&V, LINK News Bulletin, September 2008, Learning Innovation Knowledge (LINK) (www.innovationstudies.org).
- Swanson B (2009) Changing extension paradigms within a rapidly changing global economy. Paper presented at the European Seminar on Extension Education, Assisi, Italy.
- Tewodaj M, Cohen MJ, Birner R, Lemma M, Randriamamonjy J, Tadesse F, and Paulos Z (2009) Agricultural extension in Ethiopia through a gender and governance lens. IFPRI: Washington D.C.\*



# Quantification of Yield Gaps and Impact Assessment of Rice Production Technologies

### B Mondal, P Samal, NC Rath, GAK Kumar, SK Mishra, L Das, NN Jambhulkar, PK Guru, MK Bag, SM Prasad, S Roy and K Saikia

# **1. INTRODUCTION**

It is projected that there would be 60% increase in demand for agricultural production by 2050 (FAO, 2012), which is very large, but not unreachable. There is a huge 'yield gap' and closing these gaps could improve not only the productivity but also the efficiency of rice production. The term 'yield gap' has been commonly used to refer to the difference between the average farmers' yields and an estimate of a reference yield or potential yield at a specific area in a given time. Maximum attainable yield is the yield of experimental or on-farm plots with no physical, biological and economic constraints and with known management practices at a given time and in a given ecology. Potential yield (van Ittersum and Rabbinge 1997) can be defined and measured in a variety of ways such as using crop growth models, maximum yield trials, and other research experiments, or best yields from farmers' fields (Lobell et al. 2009). Farm level yield is the average farmers yield in a given area at a given time in a given ecology. Yield gaps exist because the best available production technologies are not adopted in farmers' fields which could be due to farmers' personal characteristics (e.g., lack of knowledge and skills, risk bearing ability), farm characteristics (e.g., soil quality, land slope, poor road), and unsuitability of the technology to farmers' circumstances (e.g., labour-intensive, requirement of high initial investment, poor access to inputs). Yield gap has two components, the first one cannot be narrowed or not exploitable, because it is mainly governed by the factors that are non-transferable such as environmental conditions. The second component is mainly due to difference in management practices or farmer's inefficiency level, which is manageable and can be bridged. As average crop yields are critical drivers of food prices, cropland expansion, and food security, yield gaps should be better quantified and understood (Lobell et al. 2009). An experimental technique for identifying and quantifying yield constraints in farmers' fields was developed and validated by Gomez (1977). It measures the potential yield, the actual yield, and the yields corresponding to the addition or removal of test factors over and above the farmer's levels. In agronomy, there are many crop models that can incorporate location-specific physical conditions to estimate crop growth and potential yields for particular crop types, as well as for combinations of many crops. These crop models are often developed using field and experimental data, thus providing reliable estimates of plant growth and potential yields and very much useful tool when designing agricultural systems for the maximisation of production outputs (de Koeijer et al. 1999; van Ittersum and Rabbinge 1997). However, economic, institutional and social



factors are not associated in these models (de Koeijer et al. 1999), thus preventing their usefulness in socio-economic analysis. Hence, a different approach is required that integrates the experiments in farmers field into socio-economic analysis of productive efficiency.

Impacts are the longer-term results produced by a programme or policy implementation or adoption of a technology, which may be intended and unintended, positive and negative, direct and indirect in nature. Impacts do not only refer to what has happened-in some cases, the impact is in terms of preventing negative changes; it also includes the reduction, avoidance or prevention of harm, risk, cost or other negative effects'. An impact evaluation provides evidence about the results that have been produced (or expected to be produced). It has to not only provide credible evidence that changes have occurred but also undertake credible causal inference that these changes have been at least partly due to a project, programme or technology. There are different types of impact evaluation and categorized based on the period of the exercise, like (a) ex-ante impact where evaluation undertaken before the programme is initiated or the technology being adopted; (b) ex-post impact evaluation which is conducted after a technology has been adopted by farmers in the target areas or a programme being implemented fully; and (c) concurrent impact evaluation, which gathers evidence about whether the programme is on track to deliver intended results (during implementation process). Economic evaluations combine evidence about stream of benefits and costs, through, (a) cost-benefit analysis, which transforms all the benefits (positive impacts) and costs (resources consumed and negative impacts) into monetary terms, taking into account discount factors over time, and produces a single figure of the ratio of benefits to costs, and (b) cost-effectiveness analysis, which calculates a ratio between the costs and a standardised unit of positive impacts of different propositions or choices. For impact evaluation process, the standard challenge is determining what would have happened in the absence of the programme/ technology for which evaluation is being undertaken. To understand the impact of a programme/technology on a given indicator, information would ideally be available from the beneficiaries and those same beneficiaries without the particular programme/ technology. The indicator could then be compared between these two situations to examine if the programme/technology had an impact. However, beneficiary farmers cannot be simultaneously in the project and out of the project making it necessary to search for a substitute group of farmers to act as the counterfactual - that is, what would happen in the absence of the programme/technology. To be a genuine counterfactual, they would need to be exactly like the beneficiaries, or treatment group, except they would have not received the benefit of programme/technology. Thus, any differences in the indicator could be attributed to the particular programme/ technology. Agricultural programme are generally designed to improve production or the returns to agriculture and therefore, impact evaluations of agricultural projects focus on production-based indicators such as gross margins, crop prices, yields, productivity, agricultural investment, spending on agricultural inputs, technology adoption, changes in land use patterns, crop and varietal diversification and food for home production. Collection of this type of information are challenging, beginning



with the definition of the sample unit; in fact, while production is often linked to multiple plots and crops, the decision-making process takes place at the household level.

The primary objectives of this chapter are: (a) to briefly discuss the theoretical and empirical issues related to yield gap analysis and impact assessment of modern rice production technologies; (b) to explore the existing knowledge on quantification/ factors of yield gap as well as impact assessment of agricultural technologies; and (c) to identify the gaps in knowledge, suggest research and development needs on yield gap analysis and impact assessment of agricultural technologies.

# 2. STATUS OF RESEARCH

#### 2.1. Research on rice yield gaps

**2.1.1. Approaches to quantify potential yields and yield gaps:** The factors that inhibit farmers from getting potential yields with modern varieties may be physical, economic, social, or any combinations of them. Physical conditions on some farms may prevent the farmer from exploiting the full potential of the technology. Sometimes, high yields may be physically possible but economically unprofitable. In some cases, social or institutional problems may also exist. Farmers can't acquire requisite inputs timely due to lack of credit. Further, it is also likely that the technology may not be understood by the farmers or by those directly advising them. The concept of yield gaps in crops

originated from different constraint studies carried out by International Rice Research Institute (IRRI) during seventies. To measure the potential yield, the actual yield, and the yields corresponding to the inclusion or withdrawal of test factors over and above the farmer's yield, an experimental technique was developed and validated by Gomez (1977) in plot experiments on sample farms. The relative contribution of each component to the difference between the potential yield and the farmer's yield was then assessed (Fig. 1). De Datta (1981) compared

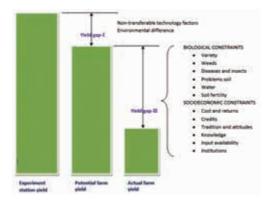


Fig. 1. The concept of yield gaps between an experiment station rice yield, the potential farm yield, and the actual farm yield (Gomez 1977).

a series of combinations of inputs of increasing intensity (management packages) to establish the yield and profitability of different combinations and to indicate the approximate intensity that is most attractive to farmers.

There are atleast four distinguished methods to estimate yield gaps at a local level (Lobell et al. 2009): (a) field experiments, (b) yield contests, (c) maximum farmer yields



based on surveys, and (d) crop model simulations. The first step associated with each method is to estimate yield ceilings (potential yield: Yp) for a given crop in a given location or region. Yield gap (Yg) is then calculated as the difference between Yp and actual yield (Ya). Although field experiments and yield contests can be used to estimate Yp for a given location and under a specific set of management practices, they require well-managed field studies in which yield-limiting and yield-reducing factors are eliminated (e.g., nutrient deficiencies, and diseases), and they must be replicated over many years to obtain a robust estimate of average Yp and their variation (Cassman et al. 2003). Field experiments and yield contests used as a basis for estimating Yp must use sowing dates and cultivar maturities that are representative of the prevailing cropping systems in the region of interest if they are to serve as benchmarks for these systems.

Surveys among farmers to estimate maximum yields from upper percentiles represent another approach to estimate Yp (Lobell et al. 2009). The best farmers' yields of a given region may give a better idea of what actually can be achieved under the normal edaphic conditions of that region (Lobell et al. 2009). It is also likely that the use of maximum farmers' yields as a proxy for potential yield is most appropriate in intensively managed cropping systems, with high levels of fertilizers and pesticides, where yield limiting factors such as nutrient deficiencies, insect attacks, diseases and competition with weeds are virtually eliminated. However, even then it is still improbable that a farmer reaches the water-limited yield potential, since optimal nutrient and pest management is quite impossible to achieve and in many cases economically not beneficial (Laborte et al. 2012). Moreover, under the conditions of family farms in the tropics, farmers often cannot afford the best available technologies. If crop production resources (including soil properties) and input levels have also been recorded, methods such as the boundary line approach or frontier analysis can be used to identify the highest yields for a given level of resource availability (Tittonell et al. 2008). However, if obstacles prevent all surveyed farmers from realizing Yp, then Yg will be underestimated. Such obstacles must operate at the same scale as the yield gap analysis and could include lack of access to inputs, lack of markets, and lack of knowledge or access to it.

Hoang (2013) has proposed a new analytical framework to examine productive efficiency in crop production systems using the economic, institutional, physical, social and technological factors of farm and the spatial heterogeneity. The novelty of this framework is the incorporation of agronomic knowledge into economic production frontier analysis. The framework has two stages; in the first stage crop growth and economic production models are used to estimate potential and best practice output levels. The framework has been applied to investigate the efficiency of rice production using district-average farm data of eight districts in Sri Lanka (Hoang, 2013). This empirical study yielded several important findings. Firstly, actual yields, on average, achieved only 60% of potential yields, leaving a 40% yield gap. This gap was decomposed into technical inefficiency (approximately 18%) and agro-economic inefficiency (approximately 22%). Theoretically, it is possible to bridge gaps between best practice and potential yields by providing optimal conditions for crop growth. In



reality, however, it might not be economically optimal for farms to bridge these gaps because the cost of marginal increments in yield might exceed the marginal gain (i.e. revenues generated from incremental yields). To overcome limitations of the above approaches, crop simulation models can be used to estimate Yp (Laborte et al. 2012). These simulation models are mathematical representations of current understanding of biophysical crop processes (phenology, carbon assimilation, assimilate partitioning) and crop responses to environmental factors. They require site-specific inputs, such as daily weather data, crop management practices (sowing date, cultivar maturity, plant density), soil properties and specification of initial conditions at sowing, such as soil water availability, and a model configuration that ensures nutrients to be nonlimiting. Grassini et al. (2015) presented an explicit rationale and methodology for selecting data sources for simulating crop yields and estimating yield gaps at specific locations that can be applied across widely different levels of data availability and quality and it was used to estimate maize yield gaps in the state of Nebraska (USA), and at a national scale for Argentina and Kenya. The aim of the suggested method was to provide a transparent, reproducible, and scientifically robust guideline for estimating yield gaps; guidelines which are also relevant for simulating the impact of climate change and land-use change at local to global spatial scales.

2.1.2. Local studies to global relevance: It is essential to compare and assess different methods of yield gap analysis across spatial scales from the field, to sub-national and national scales, to identify key components that ensure adequate transparency, accuracy, and reproducibility. Yield gap analyses for Southeast Asia helped to explain yield trends in irrigated rice and revealed that nitrogen management had to be improved to increase yields (Kropff et al. 1993). Global studies generally use empirical, statistical approaches or generic crop growth models and a grid-based approach using global datasets on climate, soils and sometimes agricultural land use and general crop calendars. The statistical methods use highest yields within a defined climatic zone (Mueller et al. 2012) or use a stochastic frontier production function (Neumann et al. 2010). They do not verify whether highest yields accurately represent the biophysical potential yield limit as confirmed by either a robust simulation model or field studies. The major limitation of this method is that it does not distinguish between irrigated and rainfed crops; thus, many yield gap estimates for a given climatic zone are based on irrigated crop yields-even in regions where the crop in question is grown almost entirely under rainfed conditions. Global studies using generic crop growth models utilize a single crop model to simulate generic crop yields for the entire globe. Often global studies using generic crop growth models do not have the explicit aim to estimate yield gaps; sometimes they aimed at estimating current yields and sensitivities of these yields to variations in management or climate (Stehfest et al. 2007).

**2.1.3. Yield gap estimates in rice:** Yield gaps in rice were observed in various countries, especially those of Asia region. Table 1 illustrates the rice yield gaps in India, Nepal, Thailand, etc. as compiled by Mondal (2011). While it was only 3.38% in China and 27.78% in India, yield gap in other countries varied from 17 to 50%. According to a study conducted by BRRI, the yield gap in rice in Bangladesh was about 1.74 t ha<sup>-1</sup> and it was estimated that at least Tk. 1260 billion could be earned from the additional





production annually by bridging the yield gap (BRRI 2011). In India, yield gap varied from 15.50 to 60% with the national average gap of 52.30% in the irrigated ecosystem (Siddiq 2000) and 2560 kg ha<sup>-1</sup> for rainfed rice (Aggarwal 2008). Nirmala and coworkers (2009) estimated 12.46% yield gap in rice in Raichur district of Karnataka, between potential yield realized at research station and the yield that was reported at the demonstration plot (yield gap I). Yield gap II, which is the difference between potential farm yield (Yd) and the actual yield (Ya) was estimated to be 11.82%. Index of yield gap, which is the ratio of the difference between potential yield (Yp) and actual yield (Ya) to the potential yield (Yp) worked out to be 22.81%. Pushpa and Srivastava (2014) quantified the gap between current and potential yields of major crops namely wheat, rice and sugarcane in eastern region of Uttar Pradesh, and identified the constraints that contribute to this yield gap. In the study area, yield gaps exist in different crops ranging up to 53% and for rice the average gap was estimated to be of 28.26%.

		Irrigated/better		
	National average	managed	Yield gap	Yield gap
Country	yield (t ha <sup>-1</sup> )	yield (t ha <sup>-1</sup> )	(t ha <sup>-1</sup> )	(%)
India	2.60	3.60	1.00	27.78
Nepal	2.50	4.20	1.70	40.47
Thailand	2.00	4.00	2.00	50.00
Vietnam	3.10	4.30	1.20	27.90
Indonesia	4.40	5.30	0.90	17.00
Philippines	2.80	3.40	0.60	17.65
China	5.70	5.90	0.20	3.38

#### Table 1. Yield levels and yield gaps in rice of several countries of Asia region.

Source: Mondal (2011)

**2.1.4. Factors causing yield gaps:** In general, factors causing yield gaps can be classified as (RAP 1999): (a) biological factors: variety, soil fertility, management practices (fertilizer, water, pest management, etc.); (b) socio-economic factors: social and economic status of farmers, family size, farm holding, knowledge and education level of farmers, contact with extension agents; (c) climatic factors: flood, drought, salinity, etc. caused by climatic changes; (d) institutional/government policy related factors: input/ output price, availability of inputs, credit supply, tenancy, etc.; and (e) factors promoting technology transfer: research-extension linkage, training of extension personnel on the new technology, their knowledge and education level about the technology, demonstration of the technology, field visits and monitoring, etc. by extension.

In a case study in Senegal (Ramaswamy and Sanders 1992), the causes of yield gaps at field scale were identified using a basic cross-correlation analysis of yield gaps against indicators of biotic and soil constraints and crop management. In fields with a low water-limited yield potential, poor soil fertility was the main factor explaining the yield gaps, while in fields with a relatively high water-limited yield potential, low soil fertility and weed infestation were the explanatory factors. Both low soil fertility and weed infestation are likely to be directly related to the low purchasing power of farmers and the resulting limited access to fertilisers and herbicides, and to the limited availability of labour on their farms. Studies from other researchers (Perez et al. 1998) in the same region mentioned water runoff as a key factor explaining observed yield gaps. Even with improved access to fertilisers and other external inputs, closing the yield gap in this region would require that farmers combine improved soil fertility and weed management with water saving techniques at field and landscape level in order to reduce production risks induced by rainfall variability, which are expected to increase with crop intensification. Pushpa and Srivastava (2014) identified the causes of yield gaps as: socio-economic, credit institutional/policy related factors, extension services and lack of improved technology. In another case study in Vietnam, Husson et al. (2004) used a similar approach as the one used in the Senegalese case study to identify the main causes of variability of upland rice yields between fields. Here, the major explanatory factors for yield differences were observed to be weed infestation and soil fertility. In central Brazil, a detailed analysis of yield variations was carried out by Affholder et al. (2003), where, the model STICS was used to simulate waterand nitrogen-limited yield for each field. A cross-correlation analysis was performed and observed that aluminium toxicity in soils, weeds and soil waterlogging were the main factors explaining the gap between observed yields and simulated water- and nitrogen limited yields.

**2.1.5. Bridging the yield gap:** Closing yield gaps to attain potential yields may be a viable option to increase the global crop production. However, traditional methods of agricultural intensification often have negative externalities. So, there is a need to explore location-specific methods of sustainable agricultural intensification. Pradhan et al. (2015) identified regions where the achievement of potential crop calorie production on currently cultivated land will meet the present and future food demand based on scenario analyses considering population growth and changes in dietary habits. By closing yield gaps in the current irrigated and rain-fed cultivated land, about 24% and 80% more crop calories can respectively be produced compared to year 2000. They have also estimated the required fertilizers (N, P<sub>2</sub>O<sub>5</sub>, and K<sub>2</sub>O) to attain the potential yields. Cui et al. (2013) achieved an increase in maize yield of 70% in an on-farm experiment by closing the yield gap and evaluated the trade-off between grain yield, nitrogen (N) fertilizer use, and GHG emissions. Based on two groups of N application experiments in six locations for 16 on-farm site-years, an integrated soilcrop system approach achieved 93% of the yield potential which is 70% higher than existing crop management. Although the N application rate increased by 38%, N<sub>2</sub>O emission intensity and the GHG intensity of the integrated system were reduced by 12% and 19%, respectively. Lobell et al. (2009) suggested that yields of 80% of its potential are an approximate of the economic optimum level. Mueller et al. (2012) presented a global-scale assessment of intensification prospects from closing 'yield gaps' (differences between observed yields and those attainable in a given region), the spatial patterns of agricultural management practices and yield limitation, and the management changes that may be necessary to achieve increased yields. They found that global yield variability is heavily controlled by fertilizer use, irrigation and climate.



Yield gaps caused by biological, socio-economic, and institutional constraints, which can be effectively addressed through an integrated crop management (1CM) practices. Transfer of the practices through extension agents could effectively help farmers to minimize yield gaps. Timely planting, irrigation, weeding, plant protection, and timely harvesting could account for more than 20% yield increase (Siddiq 2000). However, input/output prices and employment opportunities influence farmers' decision on the level of inputs to be applied.

#### 2.2. Research on impact of rice production technology/training

2.2.1. Impact of rice production technology: Based on a survey conducted in Maharashtra, India, Joshi and Bantilan (1998) observed partial and step-wise adoption of different components of the technology that range between 31% for raised-bed and furrow method of land management to 84% for improved varieties. The technology also contributes in improving the natural resource base, and eases certain women specific agricultural operations. Samal et al. (2009) assessed impact of three modern rice varieties viz. Durga, Gayatri and Sarala in the submergence prone area of Odisha state and indicated that the varieties have spread to 51% of the lowland area within three years. The returns from all the three varieties were found to be attractive in comparison to the traditional varieties in terms of additional return as well as employment generation. Wu et al. (2010) assessed the impact of improved upland rice technology on farmers' well-being using propensity-score matching technique to address the problem of 'self-selection,' because technology adoption is not randomly assigned. It applies this procedure to household survey data collected in Yunnan, China in 2000, 2002 and 2004. The findings indicated that improved upland rice technology has a robust and positive effect on farmers' well-being, as measured by income levels and the incidence of poverty. Gauhan et al. (2012) in a study in stressprone rainfed area of Nepal indicated that the yield of newer generation modern rice varieties (MVs) is not superior to that of old generation MVs despite their better adaptability in rainfed conditions. They observed through censored regression that favourable land type plays a key role in the adaptation of new generation modern varieties. In Bangladesh, Islam et al. (2012), however, mentioned that the yield of MVs and old generation MVs are not statistically different, which may explain the slow varietal replacement in Bangladesh. Similar observation were made by Behura et al. (2012) in Chattisgarh and Odisha, where the varieties released before 1990 like Swarna, Lalat and Gayatri dominate most of the area. Bagchi and Bool-Emerick (2012) observed in West Bengal that old generation MVs dominates during *aman* season while new generation MVs occupy most rice areas during boro season.

**2.2.2. Impact of training on rice production technology:** Nakano et al. (2014a) investigated impact of training provided by a large-scale private farm on the performance of surrounding small-scale rice farmers in a rain-fed area in Tanzania. They found that the training effectively enhances the adoption of improved rice cultivation practices, and profit from rice cultivation by small-holder farmers. Several other studies have shown that intensive training on rice cultivation can effectively enhance the adoption of new technologies including modern variety, chemical fertilizer



and improved agronomic practices, and productivity of rice cultivation increased both in irrigated as well as rain-fed area (Kijima et al. 2012). However, improved rice cultivation technologies are not widely adopted because of weak public extension system (Nakano et al. 2014b).

2.2.3. Impact of investment on rice research: Assessment of economic impact of new technologies delivers helpful information to justify investment efforts in research and development to generate new technologies. Kumar and Rosegrant (1994) estimated total factor productivity (TFP) of rice as 1.03%, which accounted about one-third of output growth during the period of 1971-88. The marginal returns to public investment in rice research in different regions were very high and the internal rate of return (IRR) to public investment was 55%. They have shown that, contrary to popular perception, rice research has paid handsome returns in India, even in the eastern region and demonstrated that research productivity has not declined over time. Jha and Kumar (1998) also propounded that rice research in India has been highly rewarding, generating returns that are close to 30-50% and suggested to accord high priority to three major issues: rice in eastern India, which essentially means rainfed (upland and lowland) rice; sustainable irrigated rice production (in *kharif* as well as *rabi* season); and improved efficiency in rice production. Agricultural research has contributed to breaking the seasonal barrier in rice production in India. During recent periods, area under the highly productive dry-season rice (boro) has been growing with the expansion of small-scale groundwater irrigation. Huang et al. (1998) assessed the contribution of research and technological change to the phenomenal growth in rice yield in China raising rice seeds from 2.1 t ha<sup>-1</sup> during early sixties to 6.1. t ha<sup>-1</sup> during late nineties.

# 3. KNOWLEDGE GAPS

Meeting future food demand requires a substantial increase in the yields obtained from existing crop-land. Global analyses done earlier have suggested that these gains could come from closing yield gaps - differences between yields from small-plot research versus those in farmer fields. However, closing this gap requires knowledge of causal factors not yet identified experimentally for different agro-ecological settings. Potential yields vary with the cultivars, ecology as well as the agro-climatic region. Precise knowledge on zone and ecosystem specific potential is a pre-requisite for meaningfully determining the still untapped yield of the currently popular high yielding varieties.

The impact evaluation methods and concepts has been poked with problems both, methodological, such as econometric techniques and data availability, and practical, such as ethical concerns, funding and weak incentives. Along with the sound and robust data collection methods, the impact assessment toolkit has to be evolved, particularly with regard to econometric methods. Because economic evaluation is a predictive tool, it is difficult to determine accurately what a technology's benefits and costs will be in the future. One useful and simple way of gaining insight into the impact of uncertain outcomes is a sensitivity analysis. Further, the empirical





challenge in impact assessment using observational studies is establishing a suitable counterfactual against which the impact can be measured because of self-selection problems. To accurately measure the impact of technology adoption on improving productivity of farm households, the exposure to the technology should be randomly assigned so that the effect of observable and unobservable characteristics between the treatment and comparison groups is the same, and the effect is attributable entirely to the treatment. A coherent method is desirable to identify, quantify and value the social advantages (benefits) and disadvantages (costs) in terms of common monetary units. The benefit stream over time is brought together to a net present value (NPV) by compounding or discounting. Unvalued effects/ impacts (intangibles) are described qualitatively and weighed against valued items. However, integration of this value in benefit stream is scarce or absent in existing literature about impact assessment of agricultural technologies.

# 4. RESEARCH AND DEVELOPMENT NEEDS

There are two great challenges in regard to the agriculture in India and globally: substantial increases in food demand must be met while decreasing agriculture's global environmental footprint. Closing yield gaps and increasing resource efficiency are necessary strategies towards meeting these challenges, but certainly not through unsustainable expansion of crop-land. The crucial role of nutrient and water management towards sustainable cultivation should be encouraged. Agricultural development programmes and policies must address the factors of yield limitation while emphasizing management practices that maintains trade-offs between higher production and environmental impacts. Changes to agricultural management to close yield gaps should be considered in the context of climate change scenario, which is expected to substantially impact yields and induce management adaptations.

Farmers need adequate amounts of quality inputs at the right time to obtain high yields. It is also important that the fertilizer inputs are integrated with organic manures for balanced use of nutrients. Resource-poor small but productive farmers representing more than 80% of farm population are usually unable to purchase required quantities of the inputs for better yield, therefore, they need to be supported by adequate and timely supply of credit through simplification of lending procedures and revise eligibility criteria. The action may also be taken for the expansion of rural bank branches under public sector. The coordination of research and extension is essential and the researcher should understand farmers' constraints to high productivity and accordingly develop integrated technological package (appropriate variety, timely planting, fertilizer, irrigation, and pest management) for farmers in specific locations to bridge the gaps. The extension service should ensure that the farmers apply correctly and systematically the recommended technological packages through effective training, demonstrations, field visits, monitoring, etc.

Impact evaluation provides information about actual accomplishments in the form needed by the planners/ managers/ policy makers. For evaluation of a technology, all basic data at all stages, i.e. from innovation to adoption by the end user and its' uses are necessary. Hence, proper sampling technique is to be adopted for ensuring representation of the users and the progress at various levels should be monitored. For collection of data, a suitable questionnaire should be adopted and various components such as productive, protective, environmental, etc. should be computed. Evaluation process often seeks to analyze a situation to determine why some thing happened, and suggest what might be done to correct undesirable situation. Evaluation would ideally be a continuous process, starting at appraisal, continuing with mid-term reviews and at termination, followed by an *ex-post* review, and ideally a follow-up review 5, 10 or 15 years after the end of the life of the technology.

Evaluation indicators are designed to provide a standard against which we measure or assess the progress of an activity against stated targets. They provide information and describe the state of the phenomena, that are useful to monitor changes and provide means to compare trends and progress over time. These are used as markers of the progress towards achieving short-term, intermediate term or long term objectives. It must be clear that indicators are not targets because targets are specified results in terms of quantity and/or time. The selection of indicators is of crucial which requires skill and experience. The main challenge in identifying indicators is to select those that are sufficiently representative and at the same time easy to understand and measure. It depends on the nature of the objectives and intended effects and impacts of the technology. Ideal indicators should be of: specific (clearly and unambiguously defined); measurable (either qualitatively or quantitatively); achievable (must be cost effective to monitor; result should be worth the time and money it costs to apply them); relevant (should be in consistency with the objectives and clearly reflect the goals); and time-bound (should be quite sensitive to change in the situation to be documented and sensitive to important changes such as in policy, programmes, and institutions).

Before the mid-sixties, economists gave little consideration to the distributional effects of technological changes. Hence, agricultural technologies tended to recommend or encourage technological changes which were favourable to large-scale farmers at the expense of small-scale farmers and farm labourers. In recent years, in response to the growing number of agricultural critics, agricultural scientists attempted to specifically account for some of the distributional effects of agricultural technology. Although recent attempts to account for the distributional impacts of technological change have significantly strengthened the credibility of economic models, these models still do not take into account all the distributional effects (social, economic or technical) arising from agricultural technology.

# 5. WAY FORWARD

Bridging yield gaps may not always be desirable or practical in the short term, given marginal returns for additional inputs, regional land-management policies, limits on sustainable water resources and socio-economic constraints (for example, access to capital, infrastructure, institutions and political stability). However, use of precision



agriculture techniques, conservation tillage, high-yielding hybrids, increased plant populations and multifunctional landscape management can help to mitigate negative environmental impacts of intensive agriculture. Additionally, use of organic fertilizers is also helpful for improving soil carbon, enhancing soil biota and increasing waterholding capacity. Social triggers of intensification used to differ across regions; because of development interventions by governments or NGOs, market-driven incentives for farmer investment, and land scarcity in regions which are not fully connected to global markets. Hence, to close yield gaps technological solutions must go hand in hand with lifting social and economic constraints through rights to land, critical infrastructure, and links to the world market for food and raw materials.

Impact assessment plays an important role in both identifying and communicating the implications of technology in economic terms starts from the planning process. In the early stages of research planning, preliminary partial budget assessment of the technology would assist planners and researchers in developing feasible management practices as well as to reach a consensus in priority setting of research. Once a consensus has been determined, further economic assessment will help to identify expected impacts and the implications. To be useful to the planning process, the economic implications of the particular technology/management practices must be clearly communicated. The documentation and evaluation of the strategies will generate evidences for prioritizing the technology for future research agenda. To communicate these evidences in a form meaningful for comparison, a matrix summarizing the findings of the assessment can be used. Further, impact evaluation relies on the construction of a counterfactual situation to examine the outcome of a group in two states at the same time, in and out the programme. A technology selected for the impact assessment had to demonstrate that it carefully selected a group of non-participants that were equally needy or deserving of the programme and were the same with regard to most characteristics. Finally, considering the knowledge gaps, issues and needs, the impact assessment of agricultural technologies/programmes should encompasses: establishment of proper evaluation criteria, determining distributional consideration, exact period of analysis based on economic life of the technology, identification of relevant input and output, proper valuation and discounting of inputs (costs) and outputs (benefits), and considering uncertainty and risks through sensitivity analysis.

#### References

- Affholder F, Scopel E, Madeira NJ and Capillon A (2003) Diagnosis of the productivity gap using a crop model Methodology and case study of small-scale maize production in central Brazil. Agronomie 23: 305–325.
- Aggarwal PK, Hebbar KB, Venugopalan MV, Rani S, Bala A, Biswal A and Wani SP (2008) Quantification of yield gaps in rainfed rice, wheat, cotton and mustard in India. Global Theme on Agroecosystems Report No. 43. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.
- Bagchi B and Bool-Emerick M (2012) Patterns of adoption of improved rice varieties and farmlevel impacts in stress-prone rainfed areas of West Bengal, India. In: Pandey S, Gauchan D, Malabayabas M, Bool-Emerick M, Hardy B, editors. 2012. Patterns of adoption of improved rice varieties and farm-level impacts in stress-prone rainfed areas in South Asia. Los Baños (Philippines): International Rice Research Institute.



- Behura D, Koshta A, Naik D, Samal P and Malabayabas M (2012) Patterns of adoption of improved rice varieties and farm-level impacts in stress-prone rainfed areas of Chhattisgarh and Orissa, India. In: Pandey S, Gauchan D, Malabayabas M, Bool-Emerick M, Hardy B, editors. 2012. Patterns of adoption of improved rice varieties and farm-level impacts in stress-prone rainfed areas in South Asia. Los Baños (Philippines): International Rice Research Institute.
- BRRI (2011) Study findings presented in a workshop held in May 2011 on "minimizing rice yield gap" at BRRI, Gazipur.
- Cassman KG, Dobermann A, Walters DT and Yang HS (2003) Meeting cereal demand while protecting natural resources and improving environmental quality. Annual Review of Environment and Resources 28: 315–358.
- Cui Z, Yue S, Wang G, Meng Q, Wu L, Yang Z, Zhang Q, Li S, Zhang F and Chen X (2013) Closing the yield gap could reduce projected greenhouse gas emissions: a case study of maize production in China. Global Change Biology 19: 2467–2477.
- De Datta SK (1981) Principles and practices of rice production. New York (USA): Wiley-Interscience Publications.
- de Koeijer TJ, Wossink GAA, van Ittersum MK, Struik PC and Renkema JA (1999) A conceptual model for analysing input-output coefficients in arable farming systems: From diagnosis towards design. Agricultural Systems 61: 33–44.
- FAO (2012) World agriculture towards 2030/2050: the 2012 revision. FAO, Rome.
- Gauchan D, Panta HK, Gautam S, and Nepali MB (2012) Patterns of adoption of improved rice varieties and farm-level impacts in stress-prone rainfed areas of Nepal. In: Pandey S, Gauchan D, Malabayabas M, Bool-Emerick M, Hardy B, editors. 2012. Patterns of adoption of improved rice varieties and farm-level impacts in stress-prone rainfed areas in South Asia. Los Baños (Philippines): International Rice Research Institute.
- Gomez KA (1977) On-farm assessment of yield constraints: methodological problems. In: International Rice Research Institute. Constraints to high yields on Asian rice farms: an interim report. Los Baños, Philippines.
- Grassini P, Lenny GJ, van Bussel, Justin Van Wart, Joost Wolf, Lieven C, Yang H, Boogaard H, de Groot H, Martin K van Ittersum and Cassman KG (2015) How good is good enough? Data requirements for reliable crop yield simulations and yield-gap analysis. Field Crops Research 177: 49-63.
- Hoang VN (2013) Analysis of productive performance of crop production systems: An integrated analytical framework. Agricultural Systems 116: 16-24.
- Huang J, Rozelle S and Lin JY (1998) Impacts of research and technological change on China's rice production. In: Impact of rice research. Proceedings of the International Conference on the Impact of Rice Research, 3-5 Jun 1996, Bangkok, Thailand. Thailand Development Research Institute, Bangkok, Thailand, and International Rice Research Institute, P.O. Box 933, Manila, Philippines.
- Husson O, Castella JC, Tuan HD and Naudin K (2004) Diagnostic agronomique des facteurslimitant le rendement du riz pluvial de montagnedans le nord du Vietnam. Cahiers Agricultures 13: 421–428.
- Islam MR, Islam MA, Rahman MS, Salam A, Alam S, Harun E and Bool-Emerick M (2012) Patterns of adoption of improved rice varieties and farm-level impacts in stress-prone rainfed areas of Bangladesh. In: Pandey S, Gauchan D, Malabayabas M, Bool-Emerick M, Hardy B, editors. 2012. Patterns of adoption of improved rice varieties and farm-level impacts in stress-prone rainfed areas in South Asia. Los Baños (Philippines): International Rice Research Institute.



- Jha D and Kumar P (1998) Rice production and impact of rice research in India. In: Impact of rice research. Proceedings of the International Conference on the Impact of Rice Research, 3-5 Jun 1996, Bangkok, Thailand. Thailand Development Research Institute, Bangkok, Thailand, and International Rice Research Institute, P.O. Box 933, Manila, Philippines.
- Joshi PK and Bantilan MCS (1998) Impact assessment of crop and resource management technology: a case of groundnut production technology (In En. Summaries in En, Fr.) Impact Series no.2. Patancheru 502 324, Andhra Pradesh, India: International Crops Research Institute for the Semi-Arid Tropics.
- Kijima Y, Ito Y and Otsuka K (2012) Assessing the impact of training on lowland rice productivity in an African setting: Evidence from Uganda. World Dev. 40:1610-1618.
- Kropff MJ, Cassman KG, Van Laar HH and Peng S (1993) Nitrogen and yield potential of irrigated rice. Plant and Soil 155(1): 391-394.
- Kumar P and Rosegrant MH (1994) Productivity and sources of growth for rice in India. Economic and Political Weekly 29(53): A183-A188.
- Laborte AG, De Bie CAJM, Smaling EMA, Moya PF, Boling AA and Van Ittersum MK (2012) Rice yields and yield gaps in Southeast Asia: past trends and future outlook. European Journal of Agronomy 36: 9–20.
- Lobell DB, Cassman KG and Field CB (2009) Crop yield gaps: their importance, magnitude, and causes. Annual Review of Environment and Resources 34: 1-26.
- Mondal MH (2011) Causes of yield gap and strategies for minimizing the gaps in different crops. Bangladesh Journal of Agricultural Research 36(3): 469-476.
- Mueller ND, Gerber JS, Johnston M, Ray DK, Ramankutty N and Foley JA (2012) Closing yield gaps through nutrient and water management. Nature. http://dx.doi.org/10.1038/ nature11420.
- Nakano Y, Tanaka Y and Otsuka K (2014a) To what extent do improved practices increase productivity of small-scale rice cultivation in a rain-fed area? : Evidence from Tanzania. Tokyo: National Graduate Institute for Policy Studies.
- Nakano Y, Kajisa K and Otsuka K (2014b) Credit, access to extension, and irrigation: How can we achieve rice Green Revolution in Tanzania? Tokyo: National Graduate Institute for Policy Studies.
- Neumann K, Verburg PH, Stehfest E and Müller C (2010) The yield gap of global grain production: a spatial analysis. Agricultural Systems 103: 316–326.
- Nirmala B, Deshimanya J, Muthuraman P, Meera SN and Sain M (2009) Yield gap analysis of rice in Raichur district of Karnataka. Karnataka Journal of Agricultural Sciences 22(1): 238-239.
- Perez P, Albergel J, Diatta M, Grouzis M and Sene M (1998) Rehabilitation of a semiarid ecosystem in Senegal, Farm-plot experiments. Agriculture, Ecosystems and Environment 70: 19–29.
- Pradhan P, Fischer G, van Velthuizen H, Reusser DE and Kropp JP (2015) Closing yield gaps: How sustainable can we be? PLoS ONE 10(6): e0129487.
- Pushpa and Srivastava SK (2014) Yield gap analysis and the determinants of yield gap in major crops in eastern region of Uttar Pradesh. Economic Affairs 59(4): 653-662.
- Ramaswamy S and Sanders JH (1992) Population pressure land degradation and sustainable agricultural technologies in the Sahel. Agricultural Systems 40: 361–378.
- RAP (Regional Office for Asia and the Pacific) (1999) Report of the expert consultation on bridging rice yield gap in the Asia-Pacific region. FAO.



- Samal P, Paris T and Rao KS (2009) Impact of modern rice varieties in submergence prone lowlands: a case study. Oryza 46(1): 61-66.
- Siddiq EA (2000) Bridging rice yield gap in India. In: Proceedings of Expert Conference on bridging the rice yield gap in the Asia- Pacific region, RAP, FAO. Status of oil crop production in Bangladesh, 2002. ORC, BARI.
- Stehfest E, Heistermann M, Priess JA, Ojima DS and Alcamo J (2007) Simulation of global crop production with the ecosystem model DayCent. Ecological Modelling 209: 203–219.
- Tittonell P, Shepherd KD, Vanlauwe B and Giller KE (2008) Unravelling the effects of soil and crop management on maize productivity in smallholder agricultural systems of western Kenya an application of classification and regression tree analysis. Agriculture, Ecosystems and Environment 123: 137–150.
- van Bussel LGJ, Grassini P, Van Wart J, Wolf J, Claessens L, Yang H, Boogaard H, de Groot H, Saito K, Cassman KG, Van Ittersum MK (2015) From field to atlas: upscaling of location-specific yield gap estimates. Field Crops Research 177: 98-108.
- van Ittersum MK and Rabbinge R (1997) Concepts in production ecology for analysis and quantification of agricultural input-output combinations. Field Crops Research 52: 197-208.
- Wu H, Ding S, Pandey S and Tao D (2010) Assessing the impact of agricultural technology adoption on farmers' well-being using propensity-score matching analysis in rural China. Asian Economic Journal 24: 141–160.\*



# Climate Resilient Production Technologies for Rainfed Upland Rice Systems

#### D Maiti, NP Mandal, CV Singh, SM Prasad, S Bhagat, S Roy, A Banerjee and BC Verma

#### SUMMARY

Upland rice accounts for about 13% and 13.5% respectively of world and Indian rice growing areas. It is grown under diverse topography, climatic and soil conditions, mostly as direct seeded, rainfed crop. Due to adverse topography (sloppy), poor soil conditions and absolute rain dependence, it often suffers from drought at several growth phases. Besides, poor soil nutrient conditions, biotic stresses like weeds, diseases and insects, which also are accentuated by drought, are major challenges for improving upland rice productivity. This chapter discusses progress of research to address these constraints and suggests way forward based on knowledge gap analysis.

#### **1. INTRODUCTION**

Upland rice is grown in around 15 Mha globally. Major upland rice areas are in Asia (8.9 Mha) followed by Africa (3 Mha) and Latin America (3.1 Mha). Nearly 100 million people, globally, depend on it as their daily staple food (Arraudeau 1995). In India it covers about 6.0 Mha under rainfed ecology. Major portion of upland rice areas in India are in eastern and northeastern states.

Agricultural production system of upland ecology in India is mainly monsoon dependent where rice is the major crop. However, rice productivity of the target ecology is very poor owing to several constraints including moisture stress as the most important followed by weeds, poor soil nutrient status and diseases (blast and brown spot). The upland rice ecosystem is extremely diverse as it is grown on leveled to gently rolling land (0-8% slope) and also on lands where slopes are greater than 30%. Soils vary from highly fertile volcanic and alluvial soils to highly weathered, infertile and acidic type. Upland rice is grown at altitudes of up to 2000 m above MSL and in areas with annual rainfall ranging from 1000 to 4500 mm. But erratic rainfall frequently causes drought during growth periods and results in low and unstable yields of upland rice  $(1-2 \text{ t ha}^{-1})$ , compared to irrigated lowland rice (>5 t ha^{-1}). Improving productivity of rice in the upland ecosystem is essential to meet food security needs of impoverished upland communities leading to address the set national targets of (i) Bringing Green Revolution to Eastern India (BGREI) and (ii) doubling farmers' income. In this context, the roadmap for improving rice production system of this ecology has been set through: (i) breeding for drought-tolerant, high yielding rice variety; (ii) developing climate resilient crop production technology including crop establishment, nutrient management and (ii) establishing suitable crop protection schedule (Integrated



Pest Management; IPM) including weeds, diseases and insects. All these components under compatible integration would promote improvement of rice production system of rainfed uplands. Thus, the objective of this chapter is to introduce the problems, assesses present status of research and suggest way forward for improving upland rice production system.

# 2. GENETIC ENHANCEMENT OF UPLAND RICE

Varietal improvement for upland rice at the international level has progressed independently in tropical Asia, Africa and Latin America in collaboration with different international agricultural research centers (Gupta and O'Toole 1986). Varietal improvement in Africa is mainly through international programs as there were very few national programs existed. Among the national programs, National Cereals Research Institute, Ibadan in Nigeria is the pioneer in upland rice breeding in Africa and developed many varieties, among them OS6 (FARO 11) became very popular because of its drought tolerance. The Institute has specifically developed 63 rice varieties that are low N and water use efficient, pest and diseases resistant for various rice ecologies, with the recently released upland varieties FARO 58 and 59. In the Ivory Coast the Ministry of Agricultural Research has released several useful varieties including Moroberekan, before 1966. International Agricultural Research Centers (IARCs) such as International Institute of Tropical Agriculture (IITA), IRAT (now CIRAD; the French Agricultural Research and International Cooperation Organization), WARDA (now Africa Rice) and International Rice Research Institute (IRRI) began large scale testing of new varieties in 1976. These IARCs extensively collaborated among themselves and kept developing and providing superior breeding lines to the national programs to enhance their rice production. The varietal development program in Africa revolutionized with the first NERICA (New Rice for Africa) rice variety (Jones et al. 1997) developed by the Africa Rice Center (WARDA) from a cross between the local African rice Oryza glabberima and the Asian type Oryza sativa. Total 18 NERICA varieties were released with doubled average yield in sub-Saharan Africa. Development of NERICA rice opened up new gene pools and increased rice biodiversity to scientific community.

In Latin America, upland rice improvement program was initiated in Brazil at the Instituto Agronomico Campinas (IAC), São Paulo during 1937. Since then, several important cultivars have been released. Upland rice breeding program at the National Rice and Beans Research Center (EMBRAPA/CNPAF) began in 1976 combining IAC developed varieties with African germplasm 63-83, OS6, and LAC23 to broaden genetic base and new varieties Cuiabana, Araguaia and Guarani were released between 1985-1986.

In south and south-east Asia, IRRI collaborated with national programs of different countries (Bangladesh, Cambodia, Indonesia, India, Thailand, Philippines etc.) to help in identifying more productive cultivars. In the early part, upland rice breeding in most of the countries was limited to collecting farmers' varieties, purification, and evaluation with limited hybridization. During this period many improved varieties



from tall parents were released. Upland rice breeding in the Philippines began early than any other countries and released varieties developed through hybridization. Later in the 1970s, the University of the Philippines at Los Baños developed C22, UPL Ri-3, UPL Ri-5, and UPL Ri-7 which have intermediate height, moderate tillering ability, and good grain quality. Some of these varieties were introduced in other countries though INGER program of IRRI and utilized by the breeders for varietal development under national program. Upland rice improvement program at IRRI progressed in collaboration with national programs and international institutes such as IITA, WARDA, IRAT and CIAT (Gupta and O'Toole 1986). Larger numbers of crosses were made at IRRI involving improved germplasm and traditional varieties and the lines were distributed to partners for location specific selection.

Drought tolerance breeding started with use of secondary traits for the improvement of drought tolerance in terms of grain yield under stress which met with limited success because of several limitations. From the first decade of this century the strategy changed towards direct selection for grain yield under stress for the improvement of drought tolerance. It was demonstrated at IRRI that if the stress is applied uniformly and consistently, yield under stress can be as heritable as yield under control condition and resulted in significant gain in upland rice drought tolerance (Bernier et al. 2007; Venuprasad et al. 2007; Kumar et al. 2008). For a more intensive high input upland system, a new set of cultivars were developed for Asian tropics with improved lodging resistance, harvest index and input responsiveness (Atlin et al. 2006). These cultivars combined yield potential of high yielding lowland rice with aerobic adaptation of upland rice and proved promise for water limited environment. With the standardization of screening protocol for yield under drought, large-scale conventional breeding and QTL identification programmes were started, using yield as a selection criterion. This approach has led to the successful development and release of 17 high-yielding drought-tolerant rice varieties in south Asia, south-east Asia, and Africa.

In India, systematic rice improvement program began when ICAR-National Rice Research Institute (ICAR-NRRI; formerly Central Rice Research Institute, CRRI) was established at Cuttack, Orissa in 1946. Attempts to develop drought tolerant varieties through hybridization began in Tamil Nadu and Kerala in 1955. The drought resistant variety Co31 was released from Coimbatore, Tamil Nadu but this variety has long growth duration and is photoperiod-sensitive. Initially selection from land races were made to develop drought tolerant variety in India e.g., N22 from Rajbhog in Uttar Pradesh, BR19 from Brown gora in Bihar and PTB10 from Thavala Kannan in Kerala. The All India Coordinated Rice Improvement Project (AICRIP) initiated in 1965 organized a coordinated rice improvement program involving state agricultural university/department with those of CRRI and other ICAR institutes and ushered into a new era of rice breeding. This effort resulted in identification of drought tolerant, long duration varieties like Lalnakanda41, CH45 etc. Subsequently, the focus shifted to development of shorter duration (90-110 days), drought tolerant varieties to fit in the southwest monsoon season of uplands. During next phase (1965-1970), short duration, drought tolerant varieties like Bala, Annada (Orissa), Annapurna (Kerala),



Cauvery (Tamil Nadu), Parijat (Orissa), Pusa2-21 (IARI), Rasi, Kanchan (Bihar) were developed using the semi-dwarf gene. Later on, it was realized that tall varieties are more appropriate to challenge the high weed pressure and first semi-tall variety Kalinga III was released in 1983 from CRRI. The momentum of upland rice varietal improvement programme was intensified with the establishment of Central Rainfed Upland Rice Research Station (CRURRS) of Central Rice Research Institute at Hazaribag during early 1980's. The first drought tolerant, early duration (90-95 days) variety Vandana was developed in 1992 from this station, using drought tolerant aus and high yielding indica genotypes (Sinha et al. 1994). Further efforts led to the development of varieties like Anjali (2002), Virendra (2006), CR Dhan 40 (2008) and Sahbhagidhan (2011). Simultaneous efforts at IGKV, Raipur and NDUA&T, Faizabad led to the development and release of Indira Barani dhan-1 and Shushk Samrat, respectively. The collaboration of DFID (Department of International Collaboration) with Birsa Agricultural University at Ranchi developed two popular upland varieties BVD109 and BVD110 in 2005. Subsequently, through Upland Rice Shuttle Breeding Network, linking centers in eastern and western India working on upland rice, under the aegis of ICAR-IRRI collaborative program, development of location specific upland rice varieties were initiated in 2002 leading to development of variety Sahabhagidhan (Mandal et al. 2010). The success of Sahbhagidhan due to its resilience and higher productivity under stress motivated the research group to identify QTLs that perform well under stress. Several QTLs (DTY12.1, 2.2, 3.1, 4.1, 6.1 and 9.1) were identified at IRRI. Breeding programmes at NRRI-CRURRS, Hazaribag focused on the transfer of these QTLs to productive background, in collaboration with IRRI. The first drought tolerant variety, developed through marker assisted selection (IR64 Drt1), where 2 major QTLs (qDTY2.2 and qDTY4.1) for grain yield under drought stress have been introgressed into popular variety 'IR 64' was released in 2014. Several institutions including CRRI, IRRI, BAU and DRR were involved in phenotyping and advancement of the material through multilocation testing. The Hazaribag centre has now moved on to combining drought tolerance and blast resistance genes in productive backgrounds. Vandana has been improved for drought and blast resistance with drought QTLs (12.1) and blast R gene (Pi2). The developed lines are currently under multilocation testing.

# 3. MANAGEMENT OPTIONS FOR SUSTAINABLE RICE PRODUCTION UNDER DIRECT SEEDED RAINFED ECOLOGY

Direct seeding of rice refers to the process of establishing the crop from seeds sown in the field rather than by transplanting seedlings from the nursery (Farooq et al. 2011). Upland rice is grown in rainfed unbunded fields that are prepared and seeded under dry conditions as other upland crops like maize and wheat. The upland rice ecosystem differs from other rice production systems because the plants grow in well-drained soils that are not flooded. Soils do not impound rainwater even for short period of 2-3 days because of high porosity (soil) and sloppy topography. Rice is more susceptible to drought than other crops owing to its shallow root system.



Beside direct effects, drought also declines yield through poor nitrogen (N) acquisition. Upland rice is typically a subsistence crop in India where farmers apply few or no purchased inputs (Arraudeau 1995), and do most of the work using family laborers. Joshi et al. (2013) reported that direct seeded rice (DSR) may be planted using any of the three major methods viz., dry seeding (sowing dry seeds into dry soil), wet seeding (sowing pre-germinated seeds on wet puddle soils) and water seeding (seeds sown into standing water). Dry-DSR system is traditionally practiced under rainfed uplands in most of the Asian countries.

Almost all the upland soils are low in nitrogen (N) and phosphorus (P) and have high P fixation capacity. The soils are characteristically of high porosity and of low fertility. In these soils, the productivity of the crop is not only low but also inconsistent. Next to inadequate water and nutrient supply, weeds are the major constraint in upland rice production system. Direct-seeded upland rice ecosystems are most vulnerable to weed competition. Weeds reduce upland rice grain yield and quality. Estimates of yield losses caused by weeds in upland rice range from 30 to 100%. Hence, the production technology of rainfed upland rice revolves around crop establishment, nutrient and weed management and likely shifts in weed flora due to adoption of direct-seeded rice and crop diversification.

#### 3.1. Crop establishment

To maintain good soil moisture and to maximize soil to seed contact, field should be pulverized well under conventional tillage. For zero tilled direct seeded rice (ZT-DSR), existing weeds should be burned down by using herbicides such as paraquat @ 0.5 kg a.i. ha<sup>-1</sup> or glyphosate @ 1.0 kg a.i. ha<sup>-1</sup> (Gopal et al. 2010). Soil structure is improved due to reduced compaction under zero tillage. Zero tillage beds also provide the opportunity for mechanical weed control and improved fertilizer placement.

Uneven crop stand provides less competition to weeds compared to good crop stand. Thus, ensuring good population through better land preparation and employing seed invigoration approaches may help minimizing weed population. Seed priming tools have the potential to improve emergence and stand establishment under a wide range of field condition including DSR. Recent research on a range of crop species showed faster germination, early emergence, and vigorous seedlings achieved by soaking seeds in water for some time, followed by surface drying before sowing, which may result in higher crop yield (Harris et al. 2000). In drought-prone areas, seed priming reduced the need for a high seeding rate, although it (low seed rate) can be detrimental if seeding takes place in soil that is at or near saturation (Du and Tuong 2002). Reviewed literature showed use of seed rates of up to 200 kg ha<sup>-1</sup> to grow a DSR crop. High seed rates are used mostly in areas where seed is broadcast with an aim to suppress weeds (Moody 1977). High seed rates can, on the other hand, result in large yield losses due to excessive vegetative growth before anthesis followed by a reduced rate of dry matter production after anthesis and lower foliage N concentration at heading leading to higher spikelet sterility. Moreover, dense plant populations at high seed rates can create favorable conditions for diseases and insects and make plants more prone to lodging. Under good management, optimum seed rates for row seeding 20 cm apart and broadcast were 300 and 400 seeds/m<sup>2</sup> (67.5 and 90 kg ha<sup>-1</sup> for cv. Kalinga III), respectively (Singh et al. 2017).

Seeding depth is also critical for uniform germination. Therefore, rice should not be drilled deeper than 2.5 cm to maximize uniform crop establishment (Gopal et al. 2010; Kamboj et al. 2012). Further, optimum time of planting results in improved rainwater use efficiency by 40-50% and enhances the total productivity. To optimize the use of monsoon rain, the optimum time for sowing DSR is about 10-15 days prior to onset of monsoon (Gopal et al. 2010; Kamboj et al. 2012). Singh et al. (2017) reported that an average increase of 25% grain yield when seeding date was advanced to first fortnight of June in the red sandy loam soils of uplands. They further suggested that varieties of 85-95 days would perform consistently better under advance seeding though performance of 100-105 days varieties could be subject to even distribution of rainfall during the cropping season.

#### 3.2. Nutrient management

Applying a full dose of phosphorus (P), potash (K) and one-third nitrogen (N) as basal at the time of sowing is the general recommendation for dry-DSR. Split applications of N are recommended to maximize grain yield and to reduce N losses. The remaining two-third dose of N should be applied in splits and top-dressed in equal parts at active tillering and panicle initiation stages (Kamboj et al. 2012). In addition, N application can be managed using a leaf color chart (LCC) (Kamboj et al. 2012).

Fertilizer management in direct seeded rice should aim at benefiting crop only, or if not possible, benefiting crop more than weeds. Nitrogen fertilizer is usually applied three times, at seeding, tillering, and panicle initiation stages, respectively, with a total amount from 40 (Singh and Singh 2005) up to 200 kg N ha"1 (Yang et al. 2002) split as 1/3, 1/3, 1/3 or 1/2, 1/4, 1/4 to synchronize with the demand of rice growth. Weeds must be removed before N application, otherwise a greater weed growth and competition would be created and rice yield would be even lower than when there is no N application. However, timing of N application must accurately meet demand by the rice plant, and that is often not feasible because of unpredictable rainfall. In upland conditions, N recovery can also be affected by N loss from leaching. Singh and Singh (2005) concluded that application of 1/4th N as basal in addition to two splits (1/2 at 20 and 1/4 at 40 DAS) produced more grain and straw yield of upland rice than the split application alone. When stale bed technique was adopted, application of N at the time of sowing helped in increasing the early crop vigor and rapid coverage of the field by the rice foliage with a consequent reduction in weed population (Murty et al. 1986). Mishra et al. (1995) reported that growth and vigor of both rice and weeds were less under Mussoorie Rock Phosphate at the early stage, but the growth of rice increased at the late vegetative stage compared with the single superphosphate application, without loss in grain yield. N application significantly influenced P uptake in grain and straw. Shorter duration (100–110 days) upland rice varieties were found to respond up to 18 kg applied P ha<sup>-1</sup> in lateritic soils of eastern India.



Poor phosphorus (P) availability continues to be another major constraint for agricultural productivity in drought prone rainfed uplands. On the other hand, it has been reported that the well-drained, aerobic soil conditions of this ecology support native arbuscular mycorrhiza (AM) activities which is known to promote higher P acquisition by the associated plants. AM is symbiotic association between plant roots and a special group of fungi (arbuscular mycorrhiza fungi; AMF). Sound association of upland rice with AM-fungi forming mycorrhizal symbiosis was confirmed long back by Brown et al. (1988). Partial dependence of upland rice on AMF for P acquisition was also demonstrated (Saha et al. 2005). Exploitation of natural association (AM) for improving P acquisition in upland rice was extensively attempted in upland rice at CRURRS (Hazaribag) of ICAR-NRRI and has been reviewed (Maiti 2011). Several native AM-supportive crop culture components for upland rice based cropping systems (RBCS) were identified and judicious integration of the components improved P nutrition of upland rice substantially with concomitant yield increase (Maiti et al. 2011)

#### 3.3 Weed management

Upland rice is directly sown in non-puddled, non-flooded soil, where weeds and rice germinate simultaneously. The lack of 'head start' to rice over weeds and the absence of floodwater make upland rice more weed infested than irrigated lowland rice leading to yield loss ranging between 30-98% (De Datta and Llagas, 1984). There is a diversity of floristic composition of weeds in different agro-climatic and *edaphic* conditions. A mixture of annuals and perennials, grasses and broadleaf weeds, intensifies the competitive effects of weeds in upland rice.

Mishra et al. (1995) reported from NRRI-CRURRS, Hazaribag that out of 30 weed species observed, only 14 were important based on their abundance. Among the important weeds, Echinochloa colonum was predominant in both coverage and number. The major weeds in upland, however, are Echinochloa colonum, Eleusine indica, Ageratum conyzoides, Cyperus rotundus, and Cynodon dactylon spp., Commelina benghalensis, Richardia brassilensis and Setaria glauca (Rafey and Prasad 1995). Several methods are used to control weeds in the upland rice. While direct methods are used to remove weeds completely from the upland fields; the substitutive, preventive and complimentary measures generally minimize weed populations to a manageable level. Preventive methods aiming at preventing weed dispersal and build up of seed reserves in the soil include: (1) using weed-free seeds; (2) maintaining clean fields, borders, levees and irrigation canals, and (3) cleaning farm equipment to prevent weed transfer from one field to another (De Datta and Baltazar, 1996). Good tillage and land leveling can remove weed vegetation at sowing and suppress perennial weeds; provide fine soil to allow uniform and early rice establishment; and permit uniform and easy irrigation and drainage (De Datta and Baltazar 1996).

Direct physical control methods include removal of weeds by hand, with weeding tools (hoe, scythe and spade), or with mechanical implements. Hand weeding, though effective, is expensive, labor intensive and time consuming. Besides there are difficulties



in procuring sufficient labor force for weeding operation in uplands as land preparation and transplanting operations of medium and lowland rice coincide. Mechanical weeding could be an alternative supplement to hand weeding. Hoe weedings plus hand weeding at 14 and 28 days after rice emergence respectively in upland rice has been observed to be the most suitable and economical weed-control practice.

Herbicides have the potential to offer an alternative to physical method of weed control as they are less time-consuming and cheaper. However, relative efficiency of such herbicides under upland situation is dependent upon the weed species, pattern of weed emergence, soil moisture fluctuations and correctness of land preparation. Singh et al. (2008) reported that application of pre-emergence butachlor @ 1.5 kg a.i. ha<sup>-1</sup> followed by a hand-weeding 30 DAS coupled with modified N and P application schedule proved the best strategy for integrated weed management in rainfed upland rice. Early post emergence spray of anilophos, pendimethalin, pretilachlor, pyrazosulfuron etc. has also been found effective in controlling upland weeds. However, one supplementary hand-weeding was found necessary in areas of heavy weed infestation and especially where flushes of weed emerge at intervals (Pandey and Singh 1982). Similarly, butachlor performed better when it was combined with post-emergence application of the herbicide-propanil (Carson 1975).

Herbicides have been increasingly and broadly applied in agriculture since the 1940s. However, intensive and repeated use of herbicide causes problems of environment pollution and resistant weed biotypes, which have aroused increasing concerns. Weed resistant biotypes have appeared in the major rice producing nations including China, India, Thailand and the Philippines. Reduced dependence on herbicides may bring down the costs of crop production and retard the development of herbicide resistance in weeds.

Differences in ability of rice cultivar to compete with weeds were initially reported several decades ago. Tall, droopy-leafed and vigorous traditional cultivars were reported to be more weed-competitive but lower in yield potential than short-statured, erect modern ones (De Datta 1980). Early vigor is important for the direct-seeded systems that increases stand establishment and weed competitiveness, both of which are important components for high yields in direct seeded systems (Zhao et al. 2006). Early vigor becomes more important when both rice as well as weeds emerges simultaneously. Pre-sowing seed treatments have sown good promise for promoting germination and early growth in several crops. Singh et al. (2017) reported that integration of hormonal priming by GA<sub>3</sub>@100 ppm with thermal hardening using alternate temperatures of 43/28 °C improved rice productivity by influencing growth and yield attributes of rice and reducing the weed pressure by increasing cropcompetitive ability. Intercrop systems are reported to use resources more efficiently and are able to remove more resources than mono-crop systems, thus decreasing the amount available for weed growth. Rice equivalent yield and economics indicated overall advantages accrued from intercropping of pigeon pea and cowpea with rice in 4:1 and 4:2 row ratios, respectively (Singh et al. 2017).



# 4. RICE BASED FARMING SYSTEMS FOR DROUGHT PRONE RAINFED ECOLOGY

Integrated Farming System (IFS) approach is judicious mix of two or more components while minimizing competition and maximizing complementariness with advanced agronomic management tools aimed at sustainable and environment friendly improvement of farm income and family nutrition. Integrated farming system involves preservation of bio-diversity, diversification of cropping or farming system and maximum recycling of residues. In general, farming system approach is based on several objectives that include sustainable improvement of farm house hold systems involving rural communities, enhanced input efficiency in farm production. It satisfies the basic needs of farm families, improve their nutrition and raise family income through optimum use of resources and proper recycling of residues within the system.

This approach is considered important and relevant especially for the small and marginal farmers in rainfed areas. Due to erratic monsoons and climatic variability, rainfed agriculture is becoming highly risk prone, particularly for resource poor farmers. Therefore, development and adoption of location specific IFS module would minimize risk beside other advantages. In IFS approach several components like crops, livestocks, horticultural crops, poultry, mushroom, duckery, vermin-composting, apiary, fisheries and other many enterprises get effectively integrated at the household level with a view to optimize income and resource use.

The ICAR-NRRI, Cuttack has developed modules for rainfed lowland and irrigated ecologies with rice- fish farming/ rice based farming systems. Inclusion of poultry, duckery, goatery, etc. in those IFS modules has been done. Rice based farming system including different enterprises to enhance the per unit area production of farming community is the focus of the institute.

## 5. BIOTIC STRESS MANAGEMENT STRATEGIES FOR RAINFED UPLAND

Upland rice suffers from several biotic stresses including diseases, insects and weeds. Losses due to pest (diseases and insects) range between 10-30%. Negligence of endemic areas can result in complete crop failures. Among the upland rice pests, major diseases are: rice blast (*Pyricularia oryzae*), brown spot (*Helminthosporium oryzae*/ *Bipolaris oryzae*), sheath rot (*Sarocladium oryzae*) and major insects are: yellow stem borer (*Scirpophaga incertulas*), leaf folder (*Cnaphalocrocis medinalis*), gundhi bug (*Leptocorisa acuta*) and termite (*Odontotermes obesus*) (Chauhan et al. 1991). Among important pests, this section has dealt with disease stresses which account for major loss in upland rice. The weed component has been discussed in section 3.3 of this chapter.

Rice blast caused by *Magnaporthe oryzae* is the most serious threat in uplands. Occurrences of new races of the pathogen have resulted in frequent breakdown of resistance causing 20–100% of crop losses despite utilization of many blast resistance



genes in land races (Khus and Jena 2009). Despite more than 10 decades of dedicated efforts, rice blast continues to be the most destructive disease of rice. Researchers have been successfully tapping available wild sources for many genes in rice breeding for useful traits such as blast resistance genes Pi9 from Oryza minuta, Pi-40(t) from Oryza australiensis and Pirf2-1(t) from O. rufipogon. The introgression of broadspectrum blast resistance gene(s) from Oryza rufipogon into indica rice cultivar has also been reported (Ram et al. 2007). Identification of QTLs for rice blast resistance was initiated in cv. Moroberekan, a japonica rice cultivar cultivated in Africa. 373 QTLs for blast resistance have, so far, been identified (Sharma et al. 2012). Brown spot of rice caused by *Bipolaris oryzae* (teleomorph – *Cochliobolus miyabeanus*) is another serious disease for upland rice, causing significant crop yield loss, reported to occur in all the rice growing countries. The disease has been reported to cause enormous losses in grain yield (up to 90%) particularly under epiphytotic condition as observed in Great Bengal Famine during 1942 (Ghose et al. 1960). In Indian uplands, the disease is more severe in dry/direct seeded rice in the states of Bihar, Chhatisgarh, Madhya Pradesh, Orissa, Assam, Jharkhand and West Bengal. The disease especially occurs under moisture stress combined with nutritional imbalance, particularly lack of nitrogen. The pathogen attacks the crop from seedling to milk stage. The fungus may penetrate the glumes and leave blackish spots on the endosperm.

#### 5.1. Management strategies for rice leaf blast

Among several management strategies, use of host plant resistance (HPR) is most effective. However, along with use of resistant variety, several cultural and chemical methods need to be integrated to reduce challenge of the fungus in order to decrease chances of resistance breakdown. Recently, marker assisted backcrossing (MAB) method has been extensively used for developing blast resistant variety. Several researchers (Miah et al. 2017) developed blast resistant rice varieties using MAB. Development of blast resistant upland rice varieties through conventional and molecular breeding has been discussed in the section 2 of this chapter.

Beside conventional cultural methods like burning of diseased straw and residual stubble, collection of disease free seeds, application of optimum N dose in splits, use of multilines of mixtures of several near-isogenic lines (NILs) with uniform phenotypic agricultural traits but different resistance levels can be used to control rice blast (Han et al. 2015).

The efficacy of various fungicides has been reported by researchers around the world. Variar et al. (1993b) identified suitable fungicide formulations (seed dressing with tricyclazole followed by need based spraying of ediphenphos) for controlling blast (both leaf and neck blast) in upland rice.

Recently, some botanicals were used for their antifungal activity against *M. oryzae*. Aqueous extracts of *Aloe vera, Allium sativum, Annona muricata, Azadirachta indica, Bidens pilosa, Camellia sinensis, Chrysanthemum coccineum*, processed *Coffee arabica, Datura stramonium, Nicotiana tabacum* and *Zingiber officinalis* were used by Hubert et al. (2015) for controlling rice blast disease *in-vitro* and *in-vivo*, without any phytotoxicity.



*Chaetomium cochliodes*, a biological agent, was found effective as seed dresser in the control of *M. oryzae*. Strains of *Bacillus subtillis* and *Streptomyces sindenius* have good antagonistic activity against *M. oryzae* (Yang et al. 2008).

#### 5.2. Management strategies for brown leaf spot of rice

In the past, resistance breeding efforts have been more emphasized on diseases such as blast and bacterial blight with little focus on brown spot of rice. However, efforts have been made towards searching for resistance to brown spot. Screening of upland rice germplasm revealed partial and complete resistance to the pathogen expressed in several genotypes under field conditions (Shukla et al. 1995). Bala and Goel (2006) identified resistance sources from 15 wild rice accessions with diverse origin. Subsequently, Katara et al. (2010) identified 10 QTLs, associated with brown spot resistance. Resistance breeding using conventional and molecular methods is now underway in different institutions including that of NRRI-CRURRS, Hazaribag.

Among several fungicides evaluated, seed treatment with tricyclazole (0.4%) followed by spraying with formulation of mancozeb + tricyclazole and seed treatment with thiram followed by spraying fungicide formulation of mancozeb + metalaxyl proved highly effective against brown spot. Spraying the crop with mancozeb and edifenphos also reduced disease considerably (Lore et al. 2007). Commercially available antagonistic *Pseudomonas* and *Trichoderma* species can suppress diseases by direct effect on the pathogen through mycoparasitism, antibiosis, and competition for nutrients or by improving plant immunity. Plant extracts and botanicals have also been found to be effective against brown spot disease. Aqueous leaf extract of *Thuja orientalis* proved better for minimizing the incidence of *B. oryzae* and enhancing seed germination and seeding growth (Krishnamurthy et al. 2001).

#### 5.3. Management of sheath rot of rice

Sheath rot is an important disease next to blast and brown spot for upland rice. Research on this disease under upland ecology was initiated in NRRI-CRURRS, Hazaribag during early nineties. Based on information generated on perpetuation mode and other ecological aspects of the pathogen in upland ecology, a low-cost method of managing the disease below threshold level, in short duration varieties under subsistence farming system, through mechanical separation of diseased seeds using 20% common salt solution was developed (Maiti et al. 1995). Once the infection from primary inoculums (from infected seeds) is managed the short duration varieties (90-100 days) of uplands escape the secondary infection.

#### 5.4. Integrated pest management for upland rice

Degree of pest(s) infestation, in upland rice, varies as drought occurs every year during different phenological stages of crop growth (Variar et al. 1993a). Such interactive effects necessitate modifications in the pest management schedules, depending on the intensity of drought. Considering this, a suitable IPM package with flexible options, to be implemented based on need based *ad hoc* decisions, for drought-prone upland rice was developed after validation in farmers' field (Maiti et al. 1996)



and time to time up-gradation was made based on farmers' feedback. Under favorable uplands with wet DSR, same schedule with additional component for weed management of using both early post emergence and need based post emergence (betweed 20-30 DAS) is necessary (Maiti et al. unpublished).

# 6. KNOWLEDGE GAP AND WAY FORWARD

The complex rainfed upland ecology necessitates more focus on rice varietal improvement with incorporation of multiple traits, governed by a combination of minor and major genes, into elite high yielding varieties. This can be achieved using diverse parents with desired traits following conventional pedigree breeding which is very slow process. In the current climate change scenario it is necessary to reduce the time of breeding cycle because the farmers who use varieties bred very recently are at least risk with respect to climate change. So, there is need to modernize the upland breeding program with rapid generation advancement technology, genomic prediction and recycling of new parents on the basis of field testing to increase the genetic gain. The major limitation of drought stress breeding is the lack of suitable screening methods for large volume of breeding population along with its environment specific nature. Earlier studies tried to link secondary traits with drought tolerance. However, recently direct selection for yield under drought and well-watered condition has been accepted for drought tolerance in rice (Kumar et al. 2008).

Implementation and adoption of technology related to microbial support system of nutrient management in crops is very poor owing to quality control issues. Such support system, on the other hand, is very much required for upland rice farmers which are poor and not capable of applying required amount of fertilizers in uplands. Quality control is mainly policy issue and beyond purview of research. So, exploitation of native beneficial micro-flora is an alternative to address this problem. Among microbes, fungi are most effective in acidic soils of uplands. Native AM fungi have been demonstrated to be exploited by manipulating crop culture components in favor of these fungi for improving P nutrition of upland rice (Maiti et al. 2011). Another innovative approach of exploiting native AMF flora could be through taking advantage of potential of plant species (host) to harness ecosystem services rendered by native AMF. This could be achieved by genetic manipulation of crop varieties for enhanced AM response. The agronomic and genetic manipulations for enhanced mycorrhizal nutrient acquisition and response are mutually inclusive and in combination could exploit, to the full extent, AMF biodiversity in soil. The AM-responsiveness trait, in rice, was demonstrated by the team working at NRRI-CRURRS, Hazaribag, to be linked to QTLs (Toppo et al. 2013). Identification of such QTLs would promote development of highly AM-responsive rice varieties.

By following the principles of integrated weed management system, herbicide(s) use can be reduced beside economic returns. Integrated weed management systems have the potential to reduce herbicide use (and associated costs) and to provide more robust weed management over the long term. Successful weed management is concerned with minimizing the impacts of weeds in the short term and simultaneously



ensuring yield losses under an acceptable limit in the long term because of practices being implemented. Hence, the most useful practices of weed control would provide favorable stand establishment and growth for the crop, and that are simultaneously unfavorable to the weeds through manipulation of agronomic practices like fertilizer management, cropping system and seed treatment. Due to complimentary nature of such agronomic practices, need is still felt to combine these practices to the most direct methods of weed control, including hand weeding, mechanical weeding, and herbicides. Research on rice based cropping systems has been done, so far, with the perspective of improving productivity but there has been limited research on impact of intercropping configuration on weed suppression as component of integrated weed management.

Blast and brown spot are the major diseases in uplands. These biotic stresses get accentuated under moisture stress (Variar et al. 1993a). So, genetic improvement of upland rice for blast and brown spot diseases is imperative to stabilize the production. The efficacy of blast resistance is increased when genes for partial resistance, Pi2 and Pi9 have been incorporated through MAS and haplotypes of Pi9 also have been identified from Indian landraces (Imam et al. 2016). It is necessary to develop and validate functional molecular markers for these two genes to facilitate their use in MAB. Genetics of brown spot is poorly understood. QTLs have been reported for resistance against brown spot disease but none of them explained large phenotypic variation for this trait. Three QTLs for brown spot resistance have also been identified at NRRI-CRURRS in Kalinga III/Moroberekan populations which need to be validated for future use.

The new knowledge and technologies are not reaching most of the farmers due to poor extension efforts in this area. The extension services in many countries is very poorly trained and not equipped to handle delivery of new knowledge from researchers to farmers. This lacuna should be urgently addressed. The technology delivery system should be re-oriented to handle changing circumstances and to deliver complex, knowledge-intensive technologies to farmers. There is a need to explore private sector extension agencies in commercial farming areas and other service-oriented agencies (NGOs) in food crop areas to extend new knowledge and technologies to farmers. The effectiveness of different combinations of public, private, cooperative and NGO extension agencies need to be further strengthened.

#### References

- Arraudeau MA (1995). Upland rice: Challenges and opportunities in the less favorable ecosystem. Geojournal 35: 325-328.
- Atlin GN, Lafitte H, Tao D, Laza M, Amante M and Courtois B (2006) Developing rice cultivars for high-fertility upland systems in the Asian tropics. Field Crops Research 97: 43-52.
- Bala R and Goel RK (2006) Resistance of some *Oryza* spp. Accessions to brown leaf spot (*Drechslera oryzae*) and its relationship with plant age. Plant Disease Research 21: 23-25.
- Bernier J, Kumar A, Venuprasad R, Spaner D and Atlin G (2007) A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. Crop Science 47: 507-518.



- Brown MB, Quimio TH and Castro AM (1988) Vesicular arbuscular mycorrhizas associated with upland rice (*Oryza sativa* L.). Philppine Agriculture 77: 317-332.
- Carson AG (1975) Weed competition and some promising herbicide combinations for its control in upland rice (*Oryza sativa* L.). Ghana Journal of Agricultural Science 8: 223-230.
- Chauhan VS, Chauhan JS and Variar M (1991) Current status of production and future strategy to enhance rice yields in rainfed ecosystem of plateau region of Bihar. Oryza 28: 526-331.
- De Datta SK, Llagas MA (1984) Weed problems and weed control in upland rice in tropical Asia In: An overview of upland rice research. IRRI, Los Baños, Philippines, 321-342 p.
- De Datta SK and Baltazar AM. (1996) Weed control technology as a component of rice production systems, In: Auld BA and Kim K-U (Eds.), Weed management in rice. FAO plant production and protection paper 139, Rome, 27-52 p.
- Du LV and Tuong TP (2002) Enhancing the performance of dry seeded rice: effects of seed priming, seedling rate and time of seedling, In: Pandey S, Mortimer M, Wade L, Tuong TP, Lopes K and Hardy B (eds.) Direct Seeding: Research Strategies and Opportunities. International Rice Research Institute, Los Banos, Philippines, 241–256 p.
- Farooq M, Siddique HM, Rehman H, Aziz T, Lee D-J and Wahid A (2011) Rice direct seeding: Experiences, challenges and opportunities. Soil Tillage Research 111: 87-98.
- Ghose RLM, Ghatge MB and Subramanian V (1960) Rice in India (revised edn.), New Delhi, ICAR 474 p.
- Gopal R, Jat RK, Malik RK, Kumar V, Alam MM, Jat ML, Mazid MA, Saharawat YS, McDonald A and Gupta R (2010) Direct dry seeded rice production technology and weed management in rice based systems. Technical Bulletin, International Maize and Wheat Improvement Center, New Delhi, India 28 p.
- Gupta PC and O'Toole JC (1986) Upland Rice: A Global Perspective. International Rice Research Institute. Los Banos, Laguna, Philippines 360 p.
- Han G, Lang J, Sun Y, Wang YY, Zhu YY and Lu B (2015) Intercropping of rice varieties increases the efficacy of blast control through reduced disease occurrence and variability. Journal of Integrative Agriculture 15(4): 795-802.
- Harris D, Tripathi RS and Joshi A (2000). On-farm seed priming to improve crop establishment and yield in dry direct-seeded rice, Paper presented at the Workshop on dry-seeded rice technology, Bangkok, Thailand.
- Hubert J, Mabagala RB and Mamiro DP (2015) Efficacy of selected plant extracts against *Pyricularia* grisea, causal agent of rice blast disease. American Journal of Plant Science 6: 602-611.
- Imam J, Mandal NP, Variar M and Shukla P (2016) Allele mining and selective patterns of Pi9 gene in a set of rice landraces from India. Frontiers of Plant Science 7: 1846.
- Jones MP, Dingkuhn M, Aluko GK and Semon M (1997) Inter specific *Oryza sativa* L x *O. glaberrima* Steud. progenies in upland rice improvement. Euphytica 92: 237-246.
- Kamboj BR, Kumar A, Bishnoi DK, Singla K, Kumar V, Jat ML, Chaudhary N, Jat HS, Gosain DK, Khippal A, Garg R, Lathwal OP, Goyal SP, Goyal NK, Yadav A, Malik DS, Mishra A and Bhatia R (2012) Direct Seeded Rice Technology in WesternIndo-Gangetic Plains of India: CSISA Experiences. CSISA, IRRI and CIMMYT 16 p.
- Khush GS and Jena KK (2009) Current status and future prospects for research on blast resistance in rice (*Oryza sativa L.*). In: Wang G-L, Valent B (eds) Advances in genetics, genomics and control of rice blast disease. Springer, Dordrecht.
- Krishnamurthy CD, Lokesh S and Shetty HS (2001) Occurrence, transmission and remedial aspects of *Drechslera oryzae* in paddy (*Oryza sativa* L.). Seed Research 29: 63-70.



- Kumar A, Bernier J, Verulkar S, Lafitte HR and Atlin GN (2008) Breeding for drought tolerance: direct selection for yield, response to selection and use of drought-tolerant donors in upland and lowland-adapted populations. Field Crops Research 107(3): 221-231.
- Lore JS, Thind TS, Hunjan MS and Goel RK (2007) Performance of different fungicides against multiple diseases of rice. Indian Phytopathology 60: 296-301.
- Maiti D (2011) Improving activity of native arbuscular mycorrhizal fungi (AMF) for mycorrhizal benefits in agriculture: status and prospects. Journal of Biofertilizers & Biopesticides 2: 113-117.
- Maiti, D, Toppo NN and Variar M (2011) Integration of crop rotation and arbuscular mycorrhizal (AM) fungal inoculum application for enhancing native AM activity to improve phosphorus nutrition of upland rice (*Oryza sativa* L.). Mycorrhiza 21: 659-667.
- Maiti D, Variar M and Shukla VD (1995) Integrated sheath rot management in rainfed upland rice. Oryza 32(2): 104-109.
- Maiti D, Variar M and Shukla VD (1996) Integrated Pest Management schedule for upland rice in rainfed ecosystem. Oryza 33(4): 267-272.
- Mandal NP, Sinha PK, Variar M, Shukla VD, Perraju P, Mehta A, Pathak AR, Dwivedi JL, Rathi SPS, Bhandarkar S, Singh BN, Singh DN, Panda S, Mishra NC, Singh YV, Pandya R, Singh MK, Sanger RBS, Bhatt JC, Sharma RK, Raman A, Kumar A and Atlin G (2010) Implications of genotype×input interactions in breeding superior genotypes for favorable and unfavorable rainfed upland environment. Field Crops Research 118: 135-144.
- Miah G, Rafii MY, Ismail MR, Puteh AB, Rahim HA and Latif MA (2017) Marker-assisted introgression of broad-spectrum blast resistance genes into the cultivated MR219 rice variety. Journal of the Science of Food and Agriculture 97: 2810-2818.
- Mishra GN, Shukla VD and Prasad K (1995) Weed dynamics in relation to phosphorus management in upland rice (*Oryza sativa*). Indian Journal of Agricultural Sciences 65: 746-748.
- Moody K (1977) Weed control in multiple cropping. In: Cropping Systems Research and Development for the Asian Rice Farmer, International Rice Research Institute, Los Baños, Laguna, Philippines 281-293 p.
- Murty KS, Chandra D and Srinivasulu K (1986) Upland rice environments in India and fitness of improved technologies, In: Progress in Upland Rice Research. International Rice Research Institute, Los Banos, Philippines, 25-34 p.
- Pandey J and Singh RP (1982) Weed control in upland rice in calcareous soil, In: Indian Society of Weed Science Conference Held at R.P. Krishi Viswavidyalaya, Palampur, India 8 p.
- Rafey A and Prasad K (1995) Influence of weed-management practices in pigeon pea (*Cajanas cajan*) intercropped with upland rice (*Oryza sativa*). Indian Journal of Agricultural Sciences 65: 281-282.
- Ram T, Majumder ND, Mishra B, Ansari MM, Padmavathi G (2007) Introgression of broadspectrum blast resistance gene(s) into cultivated rice (*Oryza sativa*) from wild rice O. rufipogon. Current Science 92: 225-230.
- Saha R, Saha J, Bhattacharya PM, Maiti D and Chaudhury S (2005) Arbuscular mycorrhizal responsiveness of two rice varieties in nutrient deficient laterite soil, In: Mycorrhizae (eds) Prakash Anil and Mehrotra VS. Scientific Publ. (India), Jodhpur, 21-25 p.
- Sharma TR, Rai A K, Gupta SK, Vijayan J, Devanna BN and Ray S (2012) Rice blast management through host-plant resistance: Retrospect and prospects. Agricultural Research 1(1):.37-52.
- Shukla VD, Chauhan JS, Variar M, Maiti D, Chauhan VS and Tomar JB (1995) Research of traditional rainfed rice associations to brown spot, blast and sheath rot diseases. Indian Phytopathology 48: 433-435.



- Singh CV, Ghosh BC, Mittra BN and Singh RK (2008) Integrated weed and fertilizer management for sustainable weed control and improved productivity of upland rice. Archives of Agronomy and Soil Science 54: 203-214.
- Singh CV, Mandal NP and Maiti D (2017) Upland rice technologies: A brief overview. ICAR-Central Rainfed Upland rice research station (NRRI), Hazaribag, Jharkhand, India 48 p.
- Singh RK and Singh CV (2005) Basal N fertilization increases productivity of rainfed upland rice, International Rice Research Notes 30: 33-34.
- Sinha PK, Variar M, Singh CV, Prasad K and Singh RK (1994) A new upland rice variety 'Vandana' for Bihar plateau. Indian Farming 44(3): 1-3.
- Toppo NN, Chakraborthy D, Lt. Das A, Mandal NP, Maiti D, Mandal S & Srivastava AK (2013) Identification of possible plant traits associated with arbuscular-mycorrhizal (AM) responsiveness in rice (*Oryza sativa* L.) (Abstr.), pp 155-156. In: Proc. 7<sup>th</sup> Inter. Conf. on Mycorrhiza, 6-11 January 2013, New Delhi, India 254 p.
- Variar M, Sinha PK and Chauhan VS (1993a) Interactive effect of drought on major biotic stresses in rainfed upland rice (*Oryza sativa* L.). Columban Journal of Life Sciences 1(1): 23-26.
- Variar M, Maiti D. and Shukla V D (1993b) Efficacy of fungicide formulations on management of rice blast (*Pyricularia oryzae*) in rainfed uplands. Indian Journal of Agricultural Sciences 63 (6): 386-389.
- Venuprasad R, Lafitte HR and Atlin GN (2007) Response to direct selection for grain yield under drought stress in rice. Crop Science 47: 285-293.
- Yang JH, Liu HX, Zhu GM, Pan YL, Xu LP and Guo JH (2008) Diversity analysis of antagonists from rice-associated bacteria and their application in biocontrol of rice diseases. Journal of Applied Microbiology 104(1): 91-104.
- Zhao DL, Atlin GN, Bastiaans L and Spiertz JHJ (2006) Cultivar weed competitiveness in aerobic rice: Heritability, correlated traits, and the potential for indirect selection in weed-free environment. Crop Science 46: 372-380.\*



## Acronyms

2AP	2- Acetyl-1- Pyrroline
4S4R	Self-sufficient Sustainable Seed System for Rice
ABC	Atmospheric Brown Cloud
ABTS	Antioxidant Activity
ADB	Asian Development Bank
AFLP	Amplified Fragment Length Polymorphism
AG	Anaerobic Germination
AGP	Anaerobic Germination Potential
AICRIP	All India Coordinated Rice Improvement Project
AISPA	All India Seed Producer's Association
ALEXI	Atmosphere-Land Exchange Inverse Model
ALS	Acetolactate Synthase
AMF	Arbuscular Mycorrhizal Fungi
AOSCA	Association of Seed Certification Agencies
ARC	Assam Rice Collection
AWD	Alternate Wetting and Drying
BADH	Betaine Aldehyde Dehydrogenase
BB	Bacterial Blight
BCA	Biocontrol Agent
BCM	Billion Cubic Meters
BGREI	Bringing Green Revolution to Eastern India
BHA	Butylated Hydroxyanisole
BPH	Brown Planthopper
BSP	Breeder Seed Production
CA	Conservation Agriculture
CAGR	Compound Annual Growth Rate
CAM	Crassulacean Acid Metabolism
Cas9	CRISPR associated protein-9 nuclease
CAT	Catalase
ССМ	Carbon-dioxide Concentrating Mechanisms
CGR	Crop Growth Rate
CIPK	Calcineurin-Interacting Protein Kinase
СКМ	Cubic Kilometers
CLCC	Customized LeafColour Chart
CLP	Cyclic Lipopeptides
СМ	Cubic Meters
CMS	Cytoplasmic Male Sterile

CR	Crop Residues
CRCT	Climate-Smart Resource Conservation Technology
CRISPR	Clustered Regularly Interspaced Short Palindromic Repeats
CSP	Common Symbiosis Pathway
СТ	Conventional Tillage
CVRC	Central Variety Release Committee
DAC	Department of Agriculture Cooperation
DAF	Days After Flowering
DAP	Days After Planting
DAS	Days After Sowing
DFR	Dihydro Flavonol Reductase
DH	Doubled Haploid
DisALEXI	Disaggregated ALEXI
DNA	Deoxyribonucleic Acid
DSI	Drought Susceptibility Index
DSN	Disease Screening Nursery
DSR	Direct Seeded Rice
DSSAT	Decision Support System for Agrotechnology Transfer
DUS	Distinctness, Uniformity and Stability
EBBR	Energy Balance Bowen Ratio
EBC	Energy Balance Closure
EEFs	Enhanced Efficiency Fertilizers
EGMS	Environment Sensitive Genetic Male Sterility
EI	Ecological Intensification
EPA	Environment Protection Agency
EPS	Exopolysaccharide
ER	Endoplasmic Reticulum
ERF	Ethylene Response Factors
ES	Ecosystem Services
ESV	Early Seedling Vigor
ET	Evapotranspiration
ETC	Electron Transport Chain
ETR	Electron Transport Rate
EUE	Energy Use Efficiency
FAO	Food and Agriculture Organization
FIG	Farmer Interest Group
FIRBS	Furrow Irrigated Ridge-till Bed-planting System
FLD	Front Line Demonstration
FPO	Farmer Producers' Organization



FSS	Formal Seed System
FYM	Farm Yard Manure
GA	Gibberellic Acid
GABA	Gamma-Aminobutyric Acid
GBSS I	Granule Bound Starch Synthase I
GCDT	Global Crop Diversity Trust
GCL	Glyoxylate Carboxyligase
GDC	Glycine Decarboxylase
GDH	Glycolate Gehydrogenase
GHG	Greenhouse Gas
GHGE	Greenhouse Gas Emissions
GI	Geographical Indication
GI	Glycemic Index
GIAHS	Globally Important Agriculture Heritage System
GIS	Geographic Information System
GLH	Green Leafhopper
GM	Green Manuring
GM	GeneticallyModified
GMO	GeneticallyModified Organism
GMS	Genetic Male Sterile
GOT	Grow-Out-Trail
GPC	Grain Protein Content
GPS	Global Positioning System
GSOD	Germination Stage Oxygen Deficiency
GSR	Green Super Rice
GST	Glutathione s-Transferases
GV	Granulosis Virus
GWAS	Genome-Wide Association Study
GWP	Global Warming Potential
HAR	Hypernodulation and Aberrant Root Formation
HI	Harvest Index
HLH	Helix-Loop-Helix
Но	Heterozygosity
HPR	Host Plant Resistance
HR	Homologous Recombination
HRR	Head Rice Recovery
HT	Herbicide Tolerant
HYV	High Yielding Variety
IARC	International Agricultural Research Center



IC	Internal Combustion Engine
ICAR	Indian Council of Agricultural Research
ICIA	International Crop Improvement Association
ICM	Integrated Crop Management
ICT	Information and Communication Technology
IFS	Integrated Farming System
IGP	Indo-Gangetic Plains
InDels	Insertions and Deletions
INM	Integrated Nutrient Management
IPCC	Intergovernmental Panel on Climate Change
IPM	Integrated Pest Management
IPR	Intellectual Property Right
IRAC	Insecticide Resistance Action Committee
IRC	International Rice Commission
IRDF	Integrated Rice–Duck Farming
IRRI	International Rice Research Institute
ISR	Induced Systemic Resistance
ISS	Informal Seed System
ITPGRFA	International Treaty on Plant Genetic Resources for Food and Agriculture
KRIBHCO	Krishak Bharati Cooperative
KSB	Potassium Solubilizing Bacteria
KVK	Krishi Vigyan Kendra
LAI	Leaf Area Index
LAMP	Loop-Mediated Isothermal Amplification
LB	Long Bold
LCC	Leaf Colour Chart
LD	Linkage Disequilibrium
LE	Latent Heat Flux
LR	Lime Requirement
LS	Long Slender
LTS	Long Term Storage
LUE	Light Use Efficiency
LULC	Land Use and Land Cover
LV	Low Volume
LWP	Leaf Water Potential
MAAL	Monosomic Alien Addition Lines
MAB	Marker-Assisted Backcross
MABB	Marker-Assisted Backcross Breeding
MAS	Marker Assisted Selection



MB	Medium Bold
MB	Mould Board Plough
MDH	Malate Dehydrogenase
ME	Malic Enzyme
METRIC	Mapping Evapo Transpiration at high Resolution with Internalized Calibration
MGMG	Mera Gaon Mera Gaurav
MGPT	Marker Based Genetic Purity Testing
MMT	Million Metric Tons
MoA	Ministry of Agriculture
MOU	Memorandum of Understanding
MS	Medium Slender
MSI	Membrane Stability Index
MSP	Minimum Support Price
MTA	Material Transfer Agreement
MTS	Medium Term Storage
NAS	Nicotianamine Synthase
NATCOM	National Communication
NATP	National Agricultural Technology Project
NBPGR	National Bureau of Plant Genetic Resources
NCS	National Collection from States
NCU	Neem Coated Urea
NDVI	Normalized Difference Vegetation Index
NERICA	New Rice for Africa
NGB	National Gene Bank
NGO	Non-Governmental Organization
NGR	New Generation Rice
NGS	Next Generation Sequencing
NHEJ	Non-Homologous End Joining
NIL	Near-Isogenic Line
NIR	Near Infrared Spectroscopy
NNI	Nitrogen Nutrition Index
NPT	New Plant Type
NPV	Nuclear Polyhedrosis Virus
NPV	Net Present Value
NRRI	National Rice Research Institute
NSC	National Seeds Corporation
NSP	National Seed Project
NuDSS	Nutrient Decision Support System
NUE	Nutrient Use Efficiency



ONM	Organic Nutrient Management
OSSC	Odisha State Seed Corporation
OSSOPCA	Organic Product Certification Agency
PA	Phytic Acid
PAC	Proanthcyanidins
РАН	Polycyclic Aromatic Hydrocarbons
PAM	Protospacer Adjacent Motif
PAP	Purple Acid Phosphatase
PAR	Photosynthetically Active Radiation
PCR	Polymerase Chain Reaction
PEPC	Phosphoenolpyruvate Carboxylase
PGA	Phosphoglycerate
PGMS	Photo-Sensitive Genetic Male Sterility
PGPM	Plant Growth Promoting Microbes
PGPR	Plant Growth Promoting Rhizobacteria
PGR	Plant Genetic Resources
PPDK	Pyruvate Orthophosphate Di-Kinase
РРР	Public-Private-Partnership
PSB	Phosphate Solubilizing Bacteria
PSM	Phosphate Solubilizing Microbes
PSP	Participatory Seed Production
PSTOL	Phosphorus Starvation Tolerance
PUE	Phosphorus Use Efficiency
PUL	Pullulanase
Pup1	Phosphorus Uptake 1
qPCR	Quantitative PCR
QTL	Quantitative Trait Loci
QuEFTS	Quantitative Evaluation of the Fertility of Tropical Soil
RAPD	Randomly Amplified Polymorphic DNA
RBCS	Rice Based Cropping System
RCM	Rice Crop Manger
RCT	Resource Conservation Technology
RDF	Recommended Dose of Fertilizer
RDN	Recommended Dose of Nitrogen
REY	Rice Equivalent Yield
RFLP	Restriction Fragment Length Polymorphism
RH	Relative Humidity
RIL	Recombinant Inbred Line
RKVY	Rashtriya Krishi Vikas Yojana



RM	Rice Microsatellite
RMP	Resistance Management Plan
ROS	Reactive Oxygen Species
rPGMS	Reverse Photo-Sensitive Genetic Male Sterility
RTNM	Real Time Nitrogen Management
RTV	Rice Tungro Virus
RuBisCO	Ribulose 1,5-Bisphosphate Carboxylase-Oxygenase
RuBP	Ribulose 1,5-Bisphosphate
RVC	Rice Value Chain
RWC	Relative Water Content
RWS	Rice and Wheat System
RZF	Root Zone Fertilization
SAU	State Agricultural University
SB	Short Bold
SBE	Starch Branching Enzyme
SCMR	SPAD Chlorophyll Meter Reading
SDA	State Departments of Agriculture
SDSM	Statistical Downscaling Model
SES	Standard Evaluation System
SFCI	State Farms Corporation of India
SGSV	Svalbard Global Seed Vault
ShB	Sheath Blight
SHG	Self Help Group
SMR	Seed Multiplication Ratio
SNP	Single Nucleotide Polymorphism
SOC	Soil Organic Carbon
SOD	Superoxide Dismutase
SOM	Soil Organic Matter
SQI	Soil Quality Index
SRI	System of Rice Intensification
SRR	Seed Replacement Rate
SSC	State Seed Corporation
SSD	Sub-Surface Drip
SSDC	State Seeds Development Corporations
SSFN	Site Specific Fertilizer Nitrogen
SSNM	Site Specific Nutrient Management
SSR	Simple Sequence Repeat
STARFM	Spatial and Temporal Adaptive Reflectance Fusion Model
STCR	Soil Test Crop Response



STMS	Sequence Tagged Microsatellite
Sub1	Submergence1
SVRC	State Variety Release Committee
SWAT	Soil and Water Assessment Tool
TAC	Total Anthocyanin Content
TFC	Total Flavonoid Content
TGMS	Thermo-Sensitive Genetic Male Sterility
TIL	TeQing-into-Lemont Backcross Introgression Line
TN1	Taichung Native1
TPC	Total Phenolic Content
TPR	Puddled Transplanted Rice
TSR	Tartronic Semialdehyde Reductase
ULV	Ultra Low Volume
UNDP	United Nations Development Programme
USG	Urea Super Granule
VAP	Value Added Product
VIC	Variable Infiltration Capacity
VOC	Volatile Organic Compounds
VRR	Varietal Replacement Rate
WBPH	White Backed Plant Hopper
WCE	Weed Control Efficiency
WF	Water Footprint
WOFOST	World Food Studies
WUE	Water Use Efficiency
Wx	Waxy
YSB	Yellow Stem Borer
ZT	Zero Tillage



### **Editors and Authors**

#### **Editors**

H Pathak, Director, ICAR-National Rice Research Institute, Cuttack, Odisha

AK Nayak, Head, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

M Jena, Head, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

**ON Singh**, Head, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

P Samal, Head, Social Sciences Division, ICAR-National Rice Research Institute, Cuttack, Odisha

SG Sharma, Head, Crop Physiology and Biochemistry Division, ICAR-National Rice Research Institute, Cuttack, Odisha

#### **Authors**

A Anandan, Principal Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

A Banerjee, Scientist, NRRI Regional Station Central Upland Rice Research Station, Hazaribag, Jharkhand

A Kumar, Scientist, Crop Physiology and Biochemistry Division, ICAR-National Rice Research Institute, Cuttack, Odisha

A Kumar, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

A Poonam, Principal Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

AK Mukherjee, Principal Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

AK Nayak, Head, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

Annamalai M, Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

Aravindan S, Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

**B Mondal**, Principal Scientist, Social Science Division, ICAR-National Rice Research Institute, Cuttack, Odisha

Basana Gowda G, Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

**BB Panda**, Principal Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

BC Marndi, Senior Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

BC Patra, Principal Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

BC Verma, Scientist, NRRI Regional Station Central Upland Rice Research Station, Hazaribag, Jharkhand



**BN Sadangi**, Principal Scientist and Head (Retd.), Social Science Division, ICAR-National Rice Research Institute, Cuttack, Odisha

BS Satpathy, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

C Parameswaran, Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

CV Singh, Senior Scientist, NRRI Regional Station Central Upland Rice Research Station, Hazaribag, Jharkhand

**D** Bhaduri, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

**D** Chatterjee, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

**D** Maiti, Principal Scientist and Head, NRRI Regional Station Central Upland Rice Research Station, Hazaribag, Jharkhand

Devanna BN, Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

DR Pani, Principal Scientist and Head, ICAR-NBPGR Base Centre, NRRI, Cuttack, Odisha

E Pandit, Student, Crop Improvement Division, ICAR-NRRI, Cuttack, Odisha

G Kumar, Scientist, Crop Physiology and Biochemistry Division, ICAR-National Rice Research Institute, Cuttack, Odisha

GAK Kumar, Principal Scientist, Social Science Division, ICAR-National Rice Research Institute, Cuttack, Odisha

GP Pandi G, Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

H Pathak, Director, ICAR-National Rice Research Institute, Cuttack, Odisha

HN Subudhi, DR Pani, Principal Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

J Meher, DR Pani, Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

JL Katara, Scientist, Crop Improvement Division, ICAR-National Rice research Institute, Cuttack, Odisha

JN Reddy, Principal Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

K Chakraborty, Scientist, Crop Physiology and Biochemistry Division, ICAR-National Rice Research Institute, Cuttack, Odisha

K Chattopadhay, Principal Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

K Saikia, Senior Scientist, NRRI Regional Station Regional Rainfed Lowland Rice Research Station, Gerua, Assam

K Sankari Meena, Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

KA Molla, Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

L Behera, Principal Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha



L Das, Principal Scientist, Social Science Division, ICAR-National Rice Research Institute, Cuttack, Odisha

LK Bose, Principal Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

M Azharudheen TP, Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

M Chakraborti, Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

M Debnath, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

M Jena, Head, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

M Nedunchezhianm, Principal Scientist and Head, ICAR-CTCRI Regional Station, Bhubaneswar, Odisha

M Shahid, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

MJ Baig, Principal Scientist, Crop Physiology and Biochemistry Division, ICAR-National Rice Research Institute, Cuttack, Odisha

MK Bag, Principal Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

MK Kar, Principal Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

MK Yadav, Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

MS Baite, Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

N Basak, Scientist, Crop Physiology and Biochemistry Division, ICAR-National Rice Research Institute, Cuttack, Odisha

N Umakanta, Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

**NB Patil**, Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

NC Rath, Principal Scientist, Social Science Division, ICAR-National Rice Research Institute, Cuttack, Odisha

NN Jambhulkar, Scientist, Social Science Division, ICAR-National Rice Research Institute, Cuttack, Odisha

NP Mandal, Principal Scientist, NRRI Regional Station Central Upland Rice Research Station, Hazaribag, Jharkhand

NT Borkar, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

**ON Singh**, Head, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

**P Bhattacharyya**, Principal Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

P Guru, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha



P Paneerselvam, Principal Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

P Samal, Head, Social Sciences Division, ICAR-National Rice Research Institute, Cuttack, Odisha

P Sanghamitra, Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

**P** Swain, Principal Scientist, Crop Physiology and Biochemistry Division, ICAR-National Rice Research Institute, Cuttack, Odisha

PC Rath, Principal Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

PK Hanjagi, Scientist, Crop Physiology and Biochemistry Division, ICAR-National Rice Research Institute, Cuttack, Odisha

PK Nayak, Principal Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

PK Sahu, Assistant Chief Technical Officer, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

Prabhukarthikeyan SR, Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

Prasanthi G, Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

**R Bhagawati**, Principal Scientist and Head, NRRI Regional Station Regional Rainfed Lowland Rice Research Station, Gerua, Assam

**R Khanam**, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

R Tripathi, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

Raghu S, Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

RK Sahu, Senior Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

RL Verma, Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

**RP Sah**, Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

**RR Korada**, Principal Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

S Bhagat, Senior Scientist, NRRI Regional Station Central Upland Rice Research Station, Hazaribag, Jharkhand

S Chatterjee, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

SG Sharma, Head, Crop Physiology and Biochemistry Division, ICAR-National Rice Research Institute, Cuttack, Odisha

S Lenka, Senior Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

S Mohanty, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha



S Munda, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

S Ray, Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

S Roy, Scientist, NRRI Regional Station Central Upland Rice Research Station, Hazaribag, Jharkhand

S Saha, Principal Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

S Samantaray, Principal Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

S Sarkar, Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

SC Giri, Principal Scientist, ICAR-CARI Regional Station, Bhubaneswar, Odisha

SD Mohapatra, Principal Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

Sivashankari M, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

SK Dash, Principal Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

SK Ghritlahre, Scientist, NRRI Regional Station Regional Rainfed Lowland Rice Research Station, Gerua, Assam

SK Mishra, Principal Scientist, Social Science Division, ICAR-National Rice Research Institute, Cuttack, Odisha

SK Pradhan, Principal Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

SM Prasad, Principal Scientist, NRRI Regional Station Central Upland Rice Research Station, Hazaribag, Jharkhand

SSC Pattanaik, Senior Scientist, Crop Improvement Division, ICAR-National Rice Research Institute, Cuttack, Odisha

T Adak, Scientist, Crop Protection Division, ICAR-National Rice Research Institute, Cuttack, Odisha

TK Dangar, Principal Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha

U Kumar, Scientist, Crop Production Division, ICAR-National Rice Research Institute, Cuttack, Odisha



# ICAR-National Rice Research Institute

Cuttack 753 006, Odisha, India

Phone:0671-2367768-783 (EPABX); Fax: 0671-2367757Email:director.nrri@icar.gov.in | crrictc@nic.inWebsite:www.icar-nrri.in

