M. J. Baig, P. Swain, P. S. Hanjagi, B. Gowda, P. Bhattacharyya *Editors*

Climate Change: Impact on Photosynthesis and Productivity of Rice



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Editors

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ISBN: 81-88409-09-X www.icar-nrri.in Published by Director ICAR-NRRI

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Foreword

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Rice is the staple food crop of India. It is an important global food crop providing food security to more than half of the world population. With the projected increase in world's population towards 10 billion by 2050, the demand of rice will grow even faster than for other crops. For this reason, National Rice Research Institute has set the goal of developing high yielding climate resilient varieties, technologies and improving the income and quality of life of rice farmers in India.

Several important issues cloud food security in the 21st century. In recent decades, numerous studies have attempted to project the impact of climate change on rice production. Rice ecosystems are highly diverse and pose varied challenges to the rice farmers in terms of abiotic and biotic stresses. Due to climate change, extreme abiotic factors like high and low temperatures, droughts, salinity, floods and frost damages are posing serious threats to rice photosynthesis and productivity.

The present publication brought out by the ICAR-National Rice Research Institute, Cuttack, a leading institute on rice research in the country as a part of 21 days winter school on "Climate Change : Impact on Photosynthesis and Productivity of Rice" organized from 5th to 26th November 2018 at Crop Physiology and Biochemistry Division of the institute. It's an excellent compilation providing a sound futuristic direction to climate change in general and rice photosynthesis and productivity in particular. I congratulate and compliment all those involved for their efforts in bringing out this publication

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Dr. T. Mohapatra Director General ICAR-India

Preface

Agriculture is vital for ensuring food, nutrition and livelihood security in world as well as in India. Rice is a stable food for more than 60% of Indian population. However, due to burgeoning population growth and climate change related issues agricultural production is pretend. Rice productivity also is in risk. On one side we have to increase rice yield by 60% by 2050 inorder to feed 10 billion people in 2050 and also sustain the yield in climate change related vagaries like drought, flood, submergences, high temperature, extreme events etc. Rice productivity in general and rice photosynthesis in specific is under tremendous pressure under this stress situation. In order to cope up this condition we have to synergise basic, strategic, as well as applied research on rice through innovative approach with modern techniques developed in recent times.

This book tries to bring together four themes related to rice physiology, climate change, photosynthesis and recent research advancement to sustain crop productivity in climate vagaries. Specifically themes are as follows:

- Understanding the inter-relationship of climate change-photosynthesis and rice productivity
- · Basics of rice-physiology and impacts of climate change on rice-photosynthesis
- · Rice- photosynthesis/ physiology under abiotic stresses
- Modern technologies and innovative approaches to manipulate ricephotosynthesis under changing climate

This book is a synergy of valuable contribution from renowned experts in their respective fields. It would definitely open up new thinking and approaches to solve climate change related problems in rice production in general and rice-photosynthesis in particular, and will be use full to various stakeholders including young scientists, researchers, students, academician and policy makers as well.

The editors are profusely thankful to all contributors; different published reviews in esteemed journal and books from where certain important parts are adopted and compiled. We are also thankful to Indian Council of Agricultural Research (ICAR) for providing all support for training programme from which the book is actually synthesized. We sincerely thank to Team of ICAR-National Rice Research Institute (NRRI), our students & staffs P. K. Dash, S. R. Padhy, G. K. Dash, Madhusmita Sahu and Harmohan Pradhan for providing the technical support in this publication.

Cuttack, India

M. J. Baig P. Swain P. S. Hanjagi B. Gowda G. P. Bhattacharyya



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Theme: I

Inter-relationship of climate changephotosynthesis and rice productivity



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Climate Change and Indian Agriculture from Problems to Solutions: Special reference to rice

Himanshu Pathak and Pratap Bhattacharyya

1. Introduction

In ensuring food, nutrition and livelihood security of India, agriculture plays a very crucial. Engaging almost two-third of the work force in gainful employment, agriculture accounts for a significant share in India's Gross Domestic Product. The problems of future food security and sustainability are likely to be aggravated further due to climate change and variability by putting pressure on agriculture. Recent observations showincreased snow melt and rise in sea level, rise in temperatures, increasing frequency of heat waves and heavy precipitation events. The Fifth Assessment Report of the Inter-Governmental Panel on Climate Change (IPCC) reiterated that the warming of the climate system is unequivocal.Major challenge for Indian agriculture in the context of climate change is providing food and nutritional security while limiting the greenhouse gases (GHGs) i.e., carbon dioxide (CO₂), methane (CH₄) and nitrous oxide (N₂O) emission. The task is more challengingdue to Indian farmers possessing marginal (land holdingup to 1 hectare) and small (land holding- 1- 2 hectares) land holding with meagre coping capacity accounts to more than 80%. The farms are diverse, heterogeneous and unorganized. Moreover, nearly 60% of country's net cultivated area is rainfed and exposed to various abiotic and biotic stresses.

Himanshu Pathak and Pratap Bhattacharyya ICAR-National Rice Research Institute Cuttack, Odisha



2. Impacts of Climate Change on Indian Agriculture

Climate is predominant factor in determining agricultural productivity. Over the past few decades, the human-induced changes in the climate have intensified the risk of climate-dependent crop production. The mostimminent effect of the climatic change is the rise in the atmospheric temperature due to the increased levels of GHGs in the stratosphere. It has been revealed in terms of frequent occurrence and repetition of events like droughts, melting of glaciers and rising sea levels. The quantity of rainfall and its distribution has become erratic. These changes are already demonstrative posing a serious threat to food security of the nation.

In India, 2/3rd of the cultivable land and even the irrigated system are reliant on monsoon, thereby making Indian agriculture very much prone to the risks due to climate change; especially to water stress. Flood is also a major problem in many parts of the country, especially in eastern part, where frequent episodes of flooding take place. In addition, heat waves in central and northern parts, frost in north-west and cyclone in eastern coast also causedisaster. In recent years, the frequency of these climatic extremes are getting more due to the increased atmospheric temperature, resulting in enhanced risks with substantial loss of agricultural production. Agriculture is affected by Climate change in both direct and indirect ways i.e., it affects all the component of agriculture viz., crops, soils, livestock and pests. Increase in temperature can reduce crop duration, increase crop respiration rates, alter photosynthetic process, affect the survival and distributions of pest populations and thus developing new equilibrium between crops and pest, accelerate nutrient mineralization in soils, decrease fertilizer use efficiencies, and increase evapo-transpiration. Climate change also have considerable indirect effects on agricultural land use in India due to availability of irrigation water, frequency and intensity of inter- and intra-seasonal droughts and floods, soil organic matter transformations, soil erosion, changes in pest profiles, decline in arable lands due to submergence of coastal lands, and availability of energy. The probable impacts of climate change on various sectors of Indian agriculture are listed below (Handbook of Agriculture, Indian Council of Agricultural Research, New Delhi).

2.1. Crop

Increase in ambient CO_2 is beneficial since this leads to increased photosynthesis especially in crops with C_3 mechanism of photosynthesis such as wheat and rice, and decreased evaporative losses. Despite this beneficial effects of increased ambient CO_2 , the yields of major cereals crops especially like wheat is likely to be negatively affected due to reduction in crop growth duration, increased respiration, and /or reduction in rainfall/irrigation water supplies due to rise in atmospheric temperature.

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- i. Agricultural productivity is adversely affected due to enhanced frequency and duration of extreme weather events such as flood, drought, cyclone and heat wave.
- ii. Reduction in yield in the rainfed areas due to increased crop water demand and changes in rainfall pattern during monsoon.
- iii. Declined quality of fruits, vegetables, tea, coffee, aromatic, and medicinal plants.
- iv. Alteration of agricultural pests and diseases because of more pathogen and vector development, rapid pathogen transmission and increased host susceptibility.
- Threatened agricultural biodiversity by rainfall uncertainty and temperature increase, sea level rise, and increased frequency and severity of drought, cyclones and floods.
- vi. Contrary to all the above negative impacts, cold waves and frost events in future are predicted to decreasedue to the atmospheric temperature rise, which would lead to a decreased probability of yield loss associated with frost damage in northern India in crops such as mustard and vegetables.

2.2. Impact on rice

2.2.1. Elevated CO₂ and temperature on rice production

Quantifying the effects of elevated [CO₂] and air temperature is important as they can have marked effects on rice growth and yield. In the last several decades a number of studies that have examined the effects of elevated atmospheric [CO₂] or combinations of elevated air temperature and [CO₂] on rice yield and growth (Yang et al., 2006; Sasaki et al., 2007). Most results showed that elevated [CO₂] increased yield. Conversely, several studies have shown that high air temperatures can reduce grain yield even under CO, enrichment (Horie et al., 2000, Prasad et al., 2006) owing to increased spikelet sterility (Jagadish et al., 2007). Enclosure studies with CO₂ enrichment have generally shown significant increases in rice biomass (25-40%) and yields (15-39%) at ambient temperature, but those increases tended to be offset when temperature was increased along with rising CO₂ (Moya et al., 1998). Yield losses caused by concurrent increases in CO₂ and temperature are primarily caused by high-temperature-induced spikelet sterility, but there is a lack of more detailed studies on $CO_2 \times$ temperature response curves. Increased CO_2 levels may also cause a direct inhibition of maintenance respiration at night temperatures higher than 21°C (Baker et al., 2000).

Rice response to elevated CO_2 also depends on nitrogen supply. If additional CO_2 is given when N is limited, lack of sinks for excess carbon (e.g. tillers) may limit the photosynthetic and growth response. There is evidence for genotypic variation in response to increasing CO_2 and temperature (Moya *et al.*, 1998), but as of now

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genes, traits or mechanisms associated with this have not yet been identified. In a large-scale FACE system dedicated to investigation of rice, located in northern Japan, yield increases due to elevated CO_2 (+200 µmol mol-1) averaged 7 to 15% over three years (Kim *et al.*, 2003). The increase in air temperature often offsets the stimulation of rice biomass and grain yield due to elevated [CO₂]. Only limited information on the direct effects of night temperature is available, but Morita *et al.* (2004) reported that increased night temperature alone reduced the size and weight of grain. Future studies require predicting the effect of elevated CO_2 and elevated temperature on rice growth and yield depending upon the cultivars variations, climate and soil type.

Effects of increasing minimum temperature on rice growth and yield are less understood than the effects of extremely high day temperatures on spikelet sterility. In a recent climate chamber study, there was first evidence of possible genotypic variation in resistance to high night temperatures (Counce *et al.*, 2005). Carbon losses due to growth and maintenance respiration typically amount to 40 to 60% of the total carbon fixed by a crop (Pritchard and Amthor, 2005). In tropical areas, this percentage may even be higher. According to the food and Agriculture organization of the United Nations, the net impact of projected temperature increase will be to slow the growth of rice production in Asia. Rising temperature during the past 25 years have already cut the yield growth rate by 10 - 20% in several locations.

2.2.2. Drought and rice production

It is well known that the variation of the Indian summer monsoon rainfall (June -September) from year to year has a large impact on the agricultural production and hence the economy of the country. Drought is a recurrent climatic phenomenon in India and is caused due to the country's peculiar physical and climatic characteristics as well as resulting economic and agricultural impacts. Goswami et al (2006) using a daily rainfall data set found (i) significant rising trends in the frequency and the magnitude of extreme rain events and (ii) a significant decreasing trend in the frequency of moderate events over central India during the monsoon seasons from 1951 to 2000 and predicted a substantial increase in hazards related to heavy rain over central India in the future. The rainfed regions encompassing the arid, semiarid and dry sub-humid regions are more prone to climatic variability as in these ecosystems drought is a regular part of the natural cycles affecting productivity and leading to desertification. In spite of an increased total water supply, the effects of increased precipitation variability and seasonal runoff shifts, water quality, and flood risks are likely to prevail in their impact on food production (Wassmann et al., 2009).

The frequency of droughts has varied over the decades in India. From 1899 to 1920, there were seven drought years. The incidence of drought came down between

1941 and 1965 when the country witnessed just three drought years. Again, during 1965-87, of the 21 years, 10 were drought years and the increased frequency was attributed to the El Nino Southern Oscillation (ENSO). Among the drought years, the 1987 drought was one of the worst droughts of 20th Century, with an overall rainfall deficiency of 19%. It affected 59-60% of the crop area and a population of 285 million. In 2002 too, the overall rainfall deficiency for the country as a whole was 19%. Over 300 million people spread over 18 states were affected by the drought in varying degrees. Food grains production registered the steepest fall of 29 million tonnes. Because of its semiaquatic phylogenetic origin and the diversity of rice ecosystems and growing conditions, current rice production systems rely on ample water supply and thus, are more vulnerable to drought stress than other cropping systems.

Overall, it is now well accepted that the complexity of the drought syndrome can only be tackled with a holistic approach integrating plant breeding with physiological dissection of the resistance traits and molecular genetic tools together with agronomical practices that lead to better conservation and utilization of soil moisture and matching crop genotypes with the environment (Wassmann *et al.*, 2009).

2.2.3. Flood and rice production

Rising temperatures will accelerate the rate of melting of snow and glacier ice, increasing seasonal peak flows of the Himalayan headwaters. This in turn may lead to an increased frequency of flooding particularly along the rivers whose channel capacity has been reduced by sedimentation (Aggarwal *et al.*, 2004).

Rice areas encompass a great diversity of growing conditions that vary based on the amount and duration of rainfall, depth and extent of standing water, flooding frequency, time of flooding within the growing season, soil type and topography. Crucial for survival and yield of the rice crop are the age of plants at the start of inundation, the rate of water rise, and the duration of the floods. Many parts of the tidal, deepwater and rainfed lowland rice areas are faced with abrupt increases in water level that completely inundate the crop, commonly called flash flood. These floods occur after local or remote heavy rains and may completely submerge the crop for several days with the consequent delays in development and reduced stand. India has almost all the ecologies of flood prone rice ranging from flash floods to semi deep and deepwater, where submergence occurs during early or late vegetative stage for about one to two weeks (in flash flood) and 3-6 weeks in semi deep conditions. Stagnant flooding also occurs in several parts at Bihar, Orissa, West Bengal and Assam inundating rice crops to different depth and duration and adversely affecting growth and yield. In flash flood areas, the water is invariably laden with silt which deposited on leaf surfaces causing mechanical damage and



diminishes under water photosynthesis by rice plants (Sarkar *et al.*, 2009). In deep water areas where dry direct seeding is practiced in the month of May and June, crop suffers from drought if rain is delayed after initial showers, while submergence occurs at early growth stages due to heavy rains in the month of July. In flash flood areas too, submergence and or drought could occur either alone or in combination depending upon the timing and intensity of rains causing yield penalty in both lowland and irrigated rice. Impacts are very likely to increase due to increased frequencies and intensities of some extreme weather events.

2.2.4. Rise in sea level and rice production

Climate change will also impact on rice production through rising sea level. Observations from tide gauges indicate that the mean global sea level has risen by about 10 to 25 cm over the last 100 years, and it appears that this rise was related to the rise in global mean temperature recorded over the same period. Model projections of future global mean sea level change, based on the temperature change projections, show a rise of between 13 and 94 cm by 2100, with a central estimate of 49 cm. Rice cultivation is the predominant form of land use in many coastal and deltaic regions of the tropics. No other crop apart from rice can be grown under these adverse conditions of unstable water levels and – in many locations – salinity. However, rising sea levels may reduce rice production in a sizable portion of the highly productive rice land in deltas. In the extensive deltas of tropical rivers, rising sea levels will have impacts reaching far inland. Rising sea levels in deltas areas can become major constraints to national as well as at a global rice supplies.

Sea level rise will affect the vast coastal area and flood plain zone of the Ganges-Brahmaputra and Indus Deltas. The consequences of sea level rise are (i) elevated water levels during tidal cycles and (ii) increasing cyclone frequency; both phenomena lead to increased flooding intensity as well as salt water intrusion. The combined effects of these factors will decrease agricultural production in the coastal zone of South Asia, which is dominated by rice production. A World Bank study concluded that salinity alone from a 0.3 m sea level rise will cause a net reduction of 0.5 million metric tons of rice production in Bangladesh (World Bank 2000).

3. Water

- i. Increased irrigation demands with increased temperature and higher evapotranspiration. This may also result in lowering groundwater table at some places due to over exploitation of ground water to meet out the irrigation demand.
- ii. Melting of glaciers in the Himalayas may lead to increased water availability in the Ganges, Bhramaputra and their tributaries in the short run but in the long run the availability of water would decrease considerably.
- iii. A significant increase in runoff is projected in the wet season that may lead to



increase in frequency and duration of floods and also soil erosion. However, the excess water can be harvested for future use by expanding storage infrastructure. The water balance in different parts of India is predicted to be disturbed and the quality of groundwater along the coastal track will be more affected due to intrusion of sea water.

4. Soil

- i. Reduced quantity and quality of organic matter content, which is already quite low in Indian soil.
- ii. Under elevated CO_2 concentration, crop residues have higher C:N ratio, which may reduce their rate of decomposition and nutrient supply.
- iii. Increase of soil temperature will increase N mineralization but its availability may decrease due to increased gaseous losses through processes such as volatilization and denitrification.
- iv. Change in rainfall volume and frequency and wind intensity may alter the severity, frequency and extent of soil erosion.
- v. Rise in sea level may lead to salt-water ingression in the coastal lands turning them less suitable for conventional agriculture.

5. Livestock

- i. Climate change has pronounced effect on feed production and nutrition of livestock. Increased temperature results in enhanced lignification of plant tissues and reduced digestibility. Increased water scarcity would also decrease food and fodder production.
- ii. In cooler areas, climate change has major impacts on vector-borne diseases of livestock by the expansion of vector populations. Changes in rainfall pattern may also influence expansion of vectors during wetter years, leading to large outbreaks of disease.
- iii. Global warming would increase water, shelter, and energy requirement of livestock for meeting projected milk demands.
- iv. Climate change is likely to aggravate the heat stress in dairy animals, adversely affecting their reproductive performance.

6. Fishery

- i. Increasing sea and river water temperature is likely to affect fish breeding, migration, and harvests.
- ii. Impacts of increased temperature and tropical cyclonic activity would affect the capture, production and marketing costs of the marine fish.
- iii. Coral bleaching is likely to increase due to higher sea surface temperature.

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7. Climate Resilient Technologies for Indian Agriculture

Potential adaptation strategies to deal with the impacts of climate change are developing cultivars tolerant to heat and salinity stress and resistant to flood and drought, modifying crop management practices, improving water management, adopting new farm techniques such as resource-conserving technologies, crop diversification, improving pest management, better weather forecasts and crop insurance and harnessing the indigenous technical knowledge of farmers. Some of these strategies are discussed below.

Development of new crop varieties with higher yield potential and resistant to multiple stresses (drought, flood, salinity) will be the key to maintain yield stability. Improvement of germplasm of important crops for heat tolerance should be one of the targets of breeding programmes. Similarly, it is essential to develop tolerance to multiple abiotic stresses as they occur in nature. Germplasm with greater oxidative stress tolerance may be exploited as oxidative stress tolerance, where plant's defense mechanism is targeting abiotic stresses. In addition, it is important to improve the root efficiency for the uptake of water and nutrients from soil.

Efficient use of natural resources such as water is highly critical for adaptation to climate change. With hotter temperatures and changing precipitation patterns, water will further become a scarce resource. Serious attempts towards water conservation, water harvesting and improvement of irrigation accessibility and water use efficiency will highly be essential for crop production and livelihood management. On-farm water conservation techniques, micro-irrigation systems for better water use efficiency and selection of appropriate crop need based irrigation has to be promoted. Principles of increasing water infiltration with improvement of soil aggregation, decreasing runoff with use of contours, ridges, vegetative hedges and reducing soil evaporation with use of crop residues mulch could be employed for better management of soil-water.

Small and marginal farmers having subsistence farming need assistance for making their agriculture profitable so they can improve their livelihoods and eventually help themselves escape from the ill effects of climate change. Integration should be made among crop production, livestock, agro-forestry and fish production to improve the production, income and livelihood. This is especially important for small and marginal land holding situations, which prevails in large part of the country. Major emphasis should be given on development of diverse technologies for optimization of farm resources, increased economic return, and improved sustainability in an integrated farming systems approach. New opportunities will be explored to introduce in the system to complement and synergize the productivity and income.



Crop diversification helps ameliorating the adverse effects of seasonality on family incomes and peak labor demands, reduce risk due to fluctuating monsoonal patterns, help asset improvement on farms, conserve rainwater and save irrigation water, facilitate easier weed and nutrient management, reduce water logging, and often result in better yield. Research have indicated that large areas of rice-fallows in the Indo-Gangetic Plains having adequate soil moisture can be brought under crop diversification and intensification with legumes and other crops. Diversification to other crops like pulses and oil seeds with less demand for water and nitrogen seems to be a good option for this region. Crop diversification and its associated tillage modification, crop establishment, nutrient management, and harvest practices will affect yield and soil fertility status.

The adverse impact of climate change on crop yield could be compensated with more and efficient use of plant nutrients. For example, yield reduction because of late sowing of rice as a result of delayed onset of monsoon can be compensated with higher application of N. Improved nutrient management also offers promising opportunities for mitigating GHG emission. For example, technologies including matching N supply with crop demand, using proper fertilizer formulation and right method of application, use of N-transformation inhibitors, optimizing tillage, irrigation and drainage and growing of suitable crop cultivars are some of the potential technologies to reduce N₂O emission.

Conservation agriculture and resource conservation technologies (RCTs) have proved to be highly useful to enhance resource and or input-use efficiency and provide immediate, identifiable and demonstrable economic benefits such as reductions in production costs, savings in water, fuel and labour requirements and timely establishment of crops resulting in improved yields. Yields of wheat in heat and water-stressed environments can be raised significantly by adopting the RCTs, which minimize unfavourable environmental impacts, especially in small and medium-scale farms. Zero-tillage can allow farmers to sow wheat sooner after rice harvest, so the crop heads and fills the grain before the onset of pre-monsoon hot weather.

Climate vagaries linked crop insurance schemes of both by private and public sector, should be put in place to help the farmers in reducing the risk of crop failure due to extreme climatic events. However, information is needed to frame out policies that encourage effective insurance opportunities. Micro-finance has been a success among rural poor including women. Low-cost access to financial services could be a boon for vulnerable farmers. Growing network of mobile telephony could further speed up SMS-based banking services and help farmers have better integration with financial institutions. There is a need to develop sustainable insurance system, while the rural poor are to be educated about availing such opportunities.

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Weather forecasting and early warning systems will be very useful in minimizing risks of climatic vagaries. Information and communication technologies could greatly help researchers and administrators to develop contingency plans.

8. Adaptation strategies and management practices for climate resilient rice production

8.1. Adaptation / Management options for high temperature and elevated CO,

Maximum daily temperature is very crucial because it affects the fertility of the spikelet which is the cause of decreasing yield with increasing temperature. Breeding programmes will likely offer the answer by providing new varieties that are better adapted to gradually changing conditions. Progress in rice breeding has rapidly accelerated due to the availability of the full rice genome sequence (Matsumoto *et al.*, 2005) and intensive *QTL* (quantitative trait loci) mapping efforts for a wide range of traits (Ismail *et al.* 2007). Despite the complexity of the traits associated with tolerance to most abiotic stresses, it has been found that tolerance to many of these stresses is controlled by a few *QTL*s with large effects, and that incorporation of these *QTLs* into high-yielding varieties could significantly help stabilize the yields of these varieties in stress-prone areas.

The breeding efforts for rice in hot environments currently pursue three different strategies:

- i. Physiological resilience: Tolerance to temperatures above 40°C is found in germplasm from some arid environments like Southern Iran, Pakistan or Australia.
- ii. Reproductive patterns (daytime of flowering): Rice germplasm will be screened for genotypes that flower during early morning hours (when it is still cool).
- iii. Early maturity: Genotypes with short growth durations may become pivotal for avoiding exposure to unfavourable temperatures or terminal drought in areas with short rainy seasons.

The plant breeding at the heart of adaptation measures in rice production, the efficiency of this approach can significantly be increased by other efforts, including:

- i. Geographic analysis of vulnerable regions (where the rice crop is already experiencing critical temperature levels);
- ii. Regional climate modeling to identify future "tilting points" of rice production (temperatures or CO_2 levels above which major yield losses are experienced, for example); and
- iii. Site-specific adjustment in crop management (shifting planting dates and improved water management, for example).

8.2. Adaptation /Management options for drought stress in rice

Biological measures being pursued to cope with climate variability and change include the breeding of drought-tolerant varieties and lodging-resistant cultivars in national and international rice breeding programs. Drought-tolerant rice varieties can be planted that are more adapted to warmer or drier conditions. This involves the screening and testing of rice germplasm collections that are sources of tolerance of water stress or that require less water for crop growth. Lodging-resistant cultivars will be useful in areas generally experiencing more frequent and intense typhoons with strong winds and more precipitation.

8.3. Agronomic approaches to cope with less water under irrigated rice ecosystem

The way to deal with reduced (irrigation or rain) water inflows to rice fields is to reduce the nonproductive outflows by seepage, percolation, or evaporation, while maintaining transpiration flows (as these contribute to crop growth). This can be done at land preparation, at crop establishment, and during the actual crop growth period (Bouman *et al.*, 2007).

Water saving techniques

Water saving technologies viz., Saturated soil culture, alternate wetting and drying and System of Rice Intensification can be used depending on the type and level of water scarcity, on the irrigation infrastructure (or the level of control that a farmer has over the irrigation water), and on the socioeconomics of their production environment. With absolute, or physical, water scarcity, farmers have little choice but to adapt to receiving less water than they would need to keep their fields continuously flooded.

Management options for flood stress

Flooding may come any time during the crop growing period, resulted accumulation of water on field. To overcome the situation plants need different strategies and along with the genetic development proper management also improve the tolerance capacity of the plants.

Cultivar with greater plasticity for rainfed lowland- Photoperiod sensitivity

Traditional varieties adapted to the lowland and deepwater ecosystems are generally not high yielding types, but photo-sensitive in nature. Due to the photo-sensitivity these cultivars avoid the submergence stressat the time of flowering. The possession of photo-sensitivity is significant because complete submergence during flowering even for a few days affects grain formation and spikelets become completely sterile. Besides, photoperiod-sensitive cultivars possess high plasticity and can be planted at different ages without much loss in grain yield. Bolan or double transplanting



is a traditional practice of farmers in submergence prone areas in North-East part of West Bengal, India. Use of photoperiod-sensitive cultivars has also helped in adopting this technology.

Anaerobic seeding tolerance

Direct seeding under the surface of flooded soil is known as anaerobic seeding, which requires less labour and time and comparatively less energy than transplanting. In rainfed lowland, direct and dry seeding is the common practice. If flood and or heavy rainfall occurs, due to low lying topographical condition of rainfed lowland water stagnates, establishment of rice is not proper and sometimes total area becomes barren. If rice varieties which can germinate and grow under flooded soil surface are available, the constraints for both direct wet and dry sowing would be solved and cultivation of rice could be possible with less investment.

Management strategies for sea level rise in rice

There is also steady progress in increasing the tolerance of rice to drought and salinity stress (Ismail *et al.*, 2007). Several improved drought tolerant materials are developed over recent years, including breeding lines for the rainfed lowlands. Tolerant varieties outperformed popular modern varieties by more than 50% yield under drought conditions.

9. Indian Initiatives for Developing Climate-Resilient Agriculture

India recognizes that for ensuring country's food security both in the short- and long-term and making agriculture sustainable and climate-resilient, appropriate adaptation strategies have to be developed. The country has initiated timely action to address the problems of climate change. These efforts have provided valuable inputs in terms of the regional and national level impacts of climate variability and climate change on major food grains crops, horticulture and livestock production. It has launched the National Mission for Sustainable Agriculture (NMSA) which seeks to address issues regarding sustainable agriculture, and aims at devising appropriate adaptation strategies for ensuring food security, enhanced livelihood opportunities and economic stability. The NMSA has identified 10 key dimensions for adaptation. These include improved crop seeds, livestock and fish culture; water use efficiency; pest management; improved farm practices; improved nutrient management; agricultural insurance; credit support; markets; access to information and livelihood diversification.

Building upon the early initiatives of X Five Year Plan, the Ministry of Agriculture launched the National Initiative on Climate Resilient Agriculture (NICRA) which is a flagship program of the Indian Council of Agricultural Research (ICAR) to undertake systematic long term research on the impacts and adaptation of



Indian agriculture to climate change covering not only agronomic crops but also horticulture, natural resources, livestock and fisheries. The program covering more than 21 central institutions and several state level agricultural universities is one of the largest project in any developing country. It not only addresses strategic research but also demonstration of the best bet practices on farmers' fields to cope with current variability. This has been carried out in 100 vulnerable districts of the country.

The Ministry of Agriculture through Indian Council of Agricultural Research (ICAR) has undertaken extensive capacity building of farmers, scientists and extension workers at various levels on the impact of climate change on agriculture and promotion of locally appropriate adaptation strategies. For example, in key climatically vulnerable areas of the country, the Government is promoting crop varieties tolerant to abiotic stresses, practices of improved water and nutrient management, particularly micro-irrigation, conservation agriculture, crop diversification, pest surveillance and integrated pest management. These coupled with improved agro-advisories and weather based crop insurance are likely to help farmers to cope with climate variability and minimize risks.

In rainfed agriculture, which is more risk prone and covers nearly 60% of the net sown area, the ICAR has evolved several in-situ and ex-situ water conservation technologies which are being upscaled through the Integrated Watershed Management Program (IWMP) and Mahatma Gandhi National Rural Employment Guarantee Scheme (MGNREGS). ICAR is also planning to upscale the demonstration of best bet practices through NICRA to nearly 150 districts In the country. These practices cover four modules, i.e., natural resource management, crop production, livestock and fisheries and institutional interventions.

The ICAR has also prepared a district level climate vulnerability atlas for undertaking these location specific adaptation activities both by public, private and non-governmental sector. India is also continuously working newer policy initiatives in terms of efficiently using water, energy and fertilizers in agriculture.

One of the major issues the country is facing is the inter-annual variability in rainfall and temperature affecting one part or other of the country every year. To face this challenge the ICAR has prepared District Level Contingency Plans which will be implemented at field level in the years to come.

10. Conclusion

Global climate change is considerably affecting and will continue to affect the food supply and access through direct and indirect effects on crops, soils, livestock, fisheries and pests. Therefore, concerted efforts are required for mitigation and adaptation to reduce the vulnerability of Indian agriculture to the adverse impacts



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of climate change and making it more resilient. A win-win solution is to start with such mitigation strategies that are needed for sustainable development. There is a need to develop policy framework for implementing the adaptation and mitigation options so that the farmers are saved from the adverse impacts of climate change. Development of technologies for adaptation and mitigation and their uptake at speedy rate by the farmers are essential for climate change management. Development and operationalization of adaptation strategy necessitate socio-psychological empowerment of farmers besides developing competencies in acquiring knowledge and skills related to adaptation practices.

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Crop Responses to the Rising Atmospheric CO₂: Research & Technology

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1. Introduction

Global climate changes are unique challenges to the agro-ecosystems. Human activities have altered the global atmosphere affecting the environment. The increase of human population in coming 30 years will further intensify the problem. These anthropogenic changes in atmospheric composition and climate will significantly influence the performance of crop. The exponential rise in the atmospheric CO₂ is an important aspect of global climate change, which effectively influences the productivity of the crops. Innovative approaches, for conducting long-term experiments to study the responses of crop-plants to the elevated CO₂ have been developing since 1970s. However, the majority of researches on soil-plant responses to elevated CO₂ have been done either under controlled laboratory or greenhouse conditions. Scientists have used controlled environments and field/ greenhouse based facilities to study the responses of crop and microbes to radiation, humidity, temperature and water stress. Technologies, used previously, primarily based on one component approach and have been satisfactorily worked to study the responses of plants to elevated CO₂. However, one component approach is an effective way to investigate, how one factor (such as CO₂) influences plants, in practice, but several interactions of other factors would also therefore affect the responses. Therefore, results obtained with isolated one factor experiments in plant growth chambers,

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very often suffered with the limitation of absence of organism interactions and provision of luxurious amounts of mineral nutrients. Before 1980, majority of the studies on the effects of elevated CO_2 were carried out in controlled environmental chambers including leaf cuvettes, whole plant growth chambers and small controlled greenhouses. After that, in last three decades there were introduction of precisely controlled closed systems (open top chambers) that continuously circulate the air in controlled environment of growth cabinets, phytotrons, and open field exposure systems, such as Free Air CO_2 Enrichment (FACE). However, these are moderately more expensive and less precise than closed systems. During last two decades, new approaches and computer based systematic techniques to CO_2 enrichment have potentially reduced the costs considerably. Currently, most of the studies on crop response are being carried out with the help of technologies such as FACE (Free Air CO_2 Enrichment) and OTCs (Open Top Chambers), (Figure 1).



Fig. 1 Open Top Chambers in rice field.

2. Technologies Used in CO, Enrichment Studies

Various CO_2 enrichment technologies have been developed and executed throughout the world. Working principles and design of few of them are discussed in the following section.

2.1. Leaf Cuvettes (LCs)

The 'Leaf Cuvettes' were designed for single leaf gas exchange measurements and



could be successfully used for measuring gas exchanges. The short time effect of elevated CO₂ levels on the CO₂ exchange processes in leaves could be studied by these systems. These are of two types: one is open exhaust system and another is closed circulation systems. In open exhaust system the air of known composition is passed over the leaf and change in CO, and water vapor on leaf surface is estimated as affected by leaf activities. These actually facilitate in determining the rates of photosynthesis and transpiration. Sinclair and Horie, (1989) used this cuvette system with an infrared gas analyzer (IRGA) in order to measure the changes of CO₂ concentration of air passed through the leaf. In closed circulation systems, chambers and null balance approach is used and the changes in the concentrations of CO₂ and water vapor in the chamber is determined from the rate of injection of CO₂ and H₂O required to maintain a previous fixed set point. Sinclair *et al.* (1989) designed and explained these leaf cuvettes for CO₂ exchange experiments. There, the cuvettes consisted of two disks of clear tefflon, separated by a pair of chromeplated brass rings. Leaf is inserted between monofilamental row lines in each ring. Leaf temperature is controlled with reference to ambient temperature through a water jacket in the rim of the chamber (Allen, 1994).

2.2. Sunlit Controlled Environment Chambers (SCEC)

Another controlled chamber method namely, 'Sunlit controlled environment chambers (SCEC)' was also used by scientists for measuring photosynthesis and transpiration in elevated CO_2 condition. In these chambers usually, Mylar polyester film walls were used for measuring photosynthesis and transpiration as a function of CO_2 concentration, temperature, light and soil moisture (Phenis et al., 1978).

2.3. Soil Plant Atmosphere Research (SPAR)

Soil plant atmosphere research (SPAR) systems were designed to get precise control of dry bulb temperature, CO_2 concentration and humidity of the canopy air, as well as measurements of soil water and root conditions. Jones *et al.* (1984) modified the design which actually developed by Phenis *et al.* (1978). These SPAR systems consist of sensors for measuring temperatures, IRGA for sensing CO_2 , dew point hygrometers for measuring humidity and heating devices such as heaters to regulate desired temperature. The CO_2 injection valves were used for measuring the CO_2 for photosynthesis and for cooling coil flow for regulating dew point temperature. Air in these chambers is circulated through the canopy from top to bottom and then goes out through ducts. Sensors, air sampling ports and control devices are located within the ducts, so that the air circulated to the top of the canopy had the experimentally prescribed set point of temperature, CO_2 concentration and humidity level. High light, similar to natural irradiance in variable conditions, continuous integrated measurements of CO_2 and H_2O balance, keeping root zone similar to field condition and real time tracking of ambient environment, temperature and humidity



are the advantage of this system. However, the disadvantages of this system may be listed as; chamber effects (humidity, wind gradients), limited replications and expenditure is more per unit.

2.4. Portable Field Chamber

Another system called as 'portable field chamber' based on the technique which has been actually used for canopy photosynthetic CO_2 exchange measurements. In this system non-dispersive IRGA covered with Mylar film is used for measuring CO_2 concentration. And a fan is used to circulate air within the chamber. The air is pumped through 0.6 cm diameter tube at a rate of 8 liter min-1 to IRGA in order to regulate CO_2 concentration in the chamber for measure the photosynthesis rate.

2.5. Open Top Chamber (OTC)

The OTC has been the widely used techniques for studying elevated CO₂ impact on crops and ecosystems. It facilitates field study, relatively cheaper and reliable technique. It also encourages and allows the exposure the whole plant to elevated CO₂ without any restriction. The main advantage of OTC is that it helps to maintain natural conditions to both soil and atmosphere. However, there is ample scope to improve the OTCs system for field experimentation. Open top chambers (OTCs) have been extensively used for research and education in CO₂ enrichment studies in field. This technique has been widely used in North America, South Asia, Europe in general and India, China, Srilanka in specific. Hardy and Havelka, (1975) first used a square shaped open top chamber for studying symbiotic N-fixation and photosynthesis of soybean. Rogers et al. (1983) was adapted basic cylindrical OTC system to generate large-scale CO₂ reach atmospheres in the field. Nakayama and Kimball (1988), used a square wall perforated OTC (diam, 0.2 m) made of polythene for cotton. Drake *et al.*, (1989) were developed an OTC system for CO₂ enrichment of salt marsh vegetation that could re-circulate part of input air. Uprety et al. (1998) designed, developed and implemented OTCs for South Asian climatic conditions. These chambers with frustum are being used in South Asian countries for CO_2 research network.

Uprety and his team developed the South Asian OTC system with 5 sub-systems: i) supply of pure and high concentration of CO_2 ; ii) regulator-valve-flow meter system; iii) CO_2 controlled chambers; iv) compatible high precision gas analyzer and v) software based automatic data logging and processing. Those OTCs (3-4 m diameter) were made up of aluminum frame, vertical sidewall (3 m in height) and top of the chamber was kept open to provide the near natural conditions. The chambers had a slender shape and were covered with a 0.15 mm thick transparent polyvinyl chloride (PVC) sheet. Frustums were provided at the top of the chamber to deflect air and to maintain the optimum CO_2 concentrations inside the chamber. The



cylindrical double walled plenum was used at the base for uniform CO₂ circulation. Inner side of the plenum had several small gas outlets of different sizes. Small outlets were kept near to the inlet pipe of the gas. Commercial high-pure grade CO₂ was purged at the upstream of frame to ensure proper mixing and to provide the desired CO₂ concentration. In order to distribute CO₂ enriched air uniformly into the chambers blowers were used. The CO₂ gas was supplied to the chamber from gas cylinders by a manifold system, having gas regulators and pressure gauze pipelines. Gas cylinders were fitted in a row with thick copper outlet pipe. The CO₂ gas was purged to the chambers from cylinders through manifold system with an underground pipe lines. The CO₂ was mixed with ambient air and distributed by blower into the chamber. Air blowers (preferably 30 cm diameter) were used for providing desired CO₂ concentration into chambers. Blower also helped in maintaining ambient air temperature inside the chamber. Relative photosynthetic photon flux density (PPFD) inside the chambers was maintained around 90-95%. However, gentle washing of the transparent polythene sheet (i.e outer cover of OTC) must be done frequently. Temperature and relative humidity were monitored daily in the chambers and recorded with the help of thermo hygrograph (Lawrence and Mayo, India model W/1712/Z). The diurnal variations in light intensity, temperature and CO₂ concentration both inside and outside the chambers were also recorded regularly.

2.6. Temperature Gradient Chambers (TGCs)

The 'Temperature Gradient Chambers (TGCs)' help in studying the effect of temperature on plant. Temperature Gradient Chambers were constructed for maintaining different CO₂ concentrations. Initially, the TGCs (10 m long, 50 cm height) were usually like plastic green house having provision of temperature enhancement mechanism with an air inlet at one end. The modified and improved TGC (3.6 m height x 18 m length) was like a commercial green houses with steel pipes (19 mm diameter) which are covered with 0.1 mm thick UV transparent PVC film. The TGC should be kept in north-south orientation to obtain uniform spatial distribution of direct solar beam in summer. It also helps in minimizing shading effect of neighboring chambers. For better light transparency perforated panels could be used with air regulation network. Exhaust fans (ventilation capacity of each fan, 28 m3 min-1) are essential for TGCs. As for example a TGC (2.45 m x 1.25 m x 1.25 m) was designed to study the growth and response of wheat to variable temperature gradient and CO, concentrations. In that case, the TGCs were covered with 6 mm UV stable polythene sheet, having an outlet plenum box (0.65 m x 1.25 m x 1.25 m). Fan was mounted on the roof pointing outwards as exhaust system. Temperature difference between inlet and outlet was maintained at $\pm 5^{\circ}$ C; and the temperatures were monitored by thermocouples. The IRGA was used to maintain CO₂ concentrations. Airflow (10 µmol mol-1) used to maintain



temperature gradient and it was sufficient to maintain non-significant difference of CO₂ concentration between modules.

2.7. Carbon dioxide-Temperature Gradient Chamber (CTGC)

The CTGC is used to maintain both elevated CO_2 and temperature simultaneously in the chambers. A model of CTGCs (2.5 m x 3 m x 30 m) is made off with semicircular pipe coated with zinc. The UV transparent PVC film, through which 80-85% radiations of 250-700 nm could be passed, is used to cover these chambers. Air in chamber usually heated by solar radiation, however, at low radiations, oil heaters could be used. The CO_2 was purged automatically by an electronic mass flow controller. The CO_2 with different concentration would be supplied through longitudinal pipe installed at 10 cm height at inside wall of CTGC.

2.8. Screen-Aided CO₂ Control (SACC)

The SACC is an advanced CO₂ exposure system for natural vegetation. It overcomes the microclimate associated problems with OTCs and has relatively lower operating cost. Basically each SACC system consists of a hexagonal steel frame, a transparent polycarbonate screen and an air distribution duct. Leadly and Drake (1993), first used it to study the grasslands response to elevated CO₂ in Switzerland. It was developed to enhance the incursions of outside air for reducing microclimate change in contrast to OTC. The screens were used to break the wind flow. The CO₂ dispensing rates usually higher during midday and lower at night and there exists a significant correlation between wind speed and CO, dispensing rate. However, active CO₂ control system was made such that it could maintain CO₂ concentration even in considerable windy condition. The CO₂ concentration at 25 cm above the ground surface shows that grassland vegetation acts an important buffer. Spatial control of CO₂ concentration must be good within the vegetation, so that there would be no border effect of elevated CO₂ on plant biomass. Therefore, the effect of microclimate is almost negligible in this facility and the air temperature difference is only 1K.

2.9. Free Air CO₂ Enrichment (FACE)

The FACE system provides a unique opportunity to judge the effects increase CO_2 on plant growth keeping other environmental conditions nearly undisturbed. The FACE system was developed with the following objectives; a) to maintain sufficiently steady CO_2 concentration throughout the field and study period, b) to provide reliable and precise field data on the response of crops and other plant communities, c) to develop and validate mechanistic plant growth models to predict the effects of future CO_2 concentration scenarios on plant growth and development and d) to generate original data for global carbon balance models under elevated CO_2 concentration. Maricopa FACE was the first of its kind system developed in Arizona, having arrays of 32 vertical vent pipes gas emitter ports. Each vent pipe in an array is connected to a common 22 m diameter torridly distribution plenum through an individually controlled valve. The proportional integrative differential (PID) was used to adjust gas flow, wind direction and speed and CO_2 concentration (Fig. 2).

Details of South Asian FACE

South Asian FACE system was also initiated with the same basic principle of injecting additional CO_2 in open field judiciously to maintain a predetermined elevated level of CO_2 concentration. The uniform metrological conditions of temperature, wind and humidity was maintained inside the face ring. The main components of this system were: i) the CO_2 storage and distribution component; ii) FACE ring (Plenum), through which CO_2 -enriched air is purged into target field; iii) different sensors and actuators for monitoring the environmental variations and controlling the operations and iv) an automatic electronic control system for operations. The CO_2 concentration was measured at the center of the array with the help of an IRGA. The PID valve, controls the CO_2 purging electronically (0-10 V applied for it). There was a provision of feedback signal for PID valve functioning. As for example the signal (0-5 V) corresponds to the fully closed conditions of PID valve.



Fig. 2 Schematic diagram and field mounted free air CO₂ enrichment technology (FATE and FACE)

The purging of CO_2 into FACE ring (which is adjustable) from the plenum was made at the crop canopy height. The height of the plenum could be adjusted with the growth of the targeted crop. Specially in that system the plenum could be raised up to 150 cm from ground level in steps of 5 cm. The CO_2 storage and distribution is

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done by parallel cylinders minimum of 25 numbers. Keeping a buffer tank is always advisable to maintain constant supply of CO_2 . Control system could capture every second data received from the meteorological sensors along with CO_2 concentration (by IRGA). Then the control system automatically controls the flow rate of CO_2 in the plenum with the help of PID controller valve with special developed software.

2.10. Free Air Temperature Enrichment Technology (FATE)

A typical FATE system includes, two 1500-watt IR (infra red) lamps (1.2 m above ground), which could irradiate homogeneously to 40-50 cm path of crop at an angle of 40° above ground surface (Fig-3). These lamps are regulated by proportional action-controller that could modulate the IR flux density (frequency, 10 Hz) and helps to attain target differential (T) between heated and reference. The IR lamps generally made of tungsten filament and can irradiate at about 2000°C. Importantly, the fittings are used to selectively cut off visible light. It also removes phytochrome sensitive light which helps in avoiding photo morphogenesis. Importantly, FATE irradiation must be uniform in space and time.



Fig 3. Free Air CO₂ Enrichment Technology.

3. Results of Some Case Studies in India

Open Top Chamber (OTC) was basically designed for CO_2 enrichment study in South Asian conditions to investigate the effects of elevated CO_2 in the crop plants.


The CO_2 enrichment research network of India, Bangladesh, Nepal, Pakistan and Sri Lanka for multi country, multi disciplinary experiments was first being coordinated by the National Fellow Program of the Indian Agricultural Research Institute New Delhi (Uprety *et al.*, 2000) (Fig-5). After wards a PC based system of Free Air CO_2 Enrichment (FACE) technology was also established with the help of National Physical Laboratory (NPL) New Delhi, to generate realistic biological data on the crop responses to the higher CO_2 concentration. Addition of those facilities GCTE- CO_2 Research Network (Fig-4) of India which was also dealt the vulnerable issues and adaptation strategies for meeting the rise in global food demand in the face of global environmental changes during 90s.

Few concluding results of field experiments carried out in India, under elevated CO_2 using OTC and FACE for studying the responses of crop plants at IARI under a global change national programme are listed below:

- i. Elevated CO_2 was highly significant in mitigating the adverse moisture stress effect on plant processes in Brassica species.
- ii. There is a possibility of transferring CO_2 responsive characters from one parent Brassica campestris to the hybrid Brassica oxycamp.
- iii. Studies on rice and wheat cultivars demonstrated significant increase in their growth and productivity. And those responses have been physiologically and bio-chemically characterize.
- iv. These informations are important component for decision support system for strategic choice of crop cultivars to be promoted in agricultural area vulnerable to global environmental change to sustain the livelihood in affected resource poor farmers.



Fig 4. GCTE Map CO₂ Enrichment Technology.

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Fig 5. Collaboration on CO₂ Enrichment Research in World.

4. Conclusions

Different methods of CO_2 enrichment study have their own advantages and disadvantages. However, these should also be judged with economics. Free air enrichment is the best approach for capturing real time relevant data for quantifying the impact of elevated CO_2 on crops but it is expensive, while OTCs are more amenable to the study of interactions with temperature. However, future concentrations of atmospheric CO_2 could be produced in OTCs as well as open field FACE. Moreover, the addition of temperature control system to OTCs in the field adds an important positive to the technology for climate change studies. Overall, these technologies provide an opportunity to study the future of ecosystem functioning in a CO_2 enriched warmer world. Despite few limitations, these near natural CO_2 enrichment technologies should be encouraged for further development with sophisticated electronics to counter act the challenges of future climate change research.

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Net Ecosystem Carbon Dioxide and Methane Exchanges in Rice

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1. Introduction

Net ecosystem carbon dioxide (CO_2) and methane (CH_4) exchanges between terrestrial ecosystems and atmosphere is regarded as one of the major processes that potentially affect atmospheric greenhouse gas (GHG) concentration (Bhattacharyya *et al.*, 2014, 2016). Net ecosystem CO_2 exchange (NEE) follows two different sign conventions for studying GHGs flux dynamics under ecosystem ecology: (a) positive sign denotes a net flux of CO_2 towards the atmosphere from vegetation (crop) canopy (i.e. source), whereas, (b) negative sign indicates a net uptake of CO_2 by the vegetation-soil system (i.e. sink) (Bhattacharyya *et al.* 2014, Swain *et al.* 2016). Likewise for net ecosystem CH_4 exchange (NEME) in soil can act as source and/or sink for CH_4 (Swain *et al.*, 2016, 2018). Quantifying GHGs emission for accounting GHGs budget and in order to predict future emissions in changing climatic scenario, GHGs fluxes are being assessed globally in varied ecosystem both short and long term basis (Bhattacharyya *et al.*, 2016).

Atmospheric GHGs are responsible for global warming by trapping and re-radiating the long wave radiation towards earth surface, resulting into warming of the earth atmosphere (Bhattacharyya *et al.* 2016). The concentrations of atmospheric CO₂, CH₄ and nitrous oxide (N₂O) have increased by ~40%, 150% and 20%, respectively since beginning of the industrial era (Tian *et al.* 2016). Global mean surface temperature has increased by a 0.85°C in last ~100 years. Frequency and intensity of the extreme events viz. cyclone, heat and cold waves, frost days etc. are likely

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to be increased in coming decades as anticipated by various climate change models (IPCC, 2014, 2018). Carbon dioxide is the major GHG that contributes to global warming, followed by CH_4 and N_2O , altogether responsible for anthropogenic climate change (Tian *et al.* 2016). Agriculture contributes to CO_2 emissions through landuse changes, CH_4 emission mostly from submerged rice cultivation and N_2O emission due to application of nitrogenous fertilizers (Bhattacharyya *et al.* 2016). Moreover, current projection of the increase of CO_2 concentration in atmosphere is ~700-1000 ppm by the end of this century (IPCC, 2014). It has been found that global warming poses serious threat to crop production (Lobell and Asner, 2003).

Submerged rice cultivation is regarded as one of the major sources of CH₄ and has a role to play in the global CH₄ budget. Agriculturally important trace gas CH₄ is having global warming potential (GWP) of 25, in 100-year time scale (IPCC, 2014). Natural and anthropogenic wetlands (e.g. submerged rice-paddy), likely the largest natural source of CH₄ to the atmosphere, accounts for ~20-50% of its emissions on a global scale (Ciais et al. 2013). Soil is the primary site, where both CH₄ production and oxidation takes place. Methanogenic archaea under anoxic conditions are responsible for CH₄ production (methanogenesis) (Trotsenko and Khmelenina, 2002; Bhattacharyya et al. 2016). Methanogenesis occurs due to microbial decomposition of organic matter under the anaerobic conditions created by prolonged submergence of rice field (Rydin and Jeglum, 2006). Methanotrophic bacteria are instrumental in CH₄ oxidation driving absorption of atmospheric CH₄ in soil. Carbon dioxide is released largely from microbial decay of plant litter and soil organic matter decomposition during rice cultivation (Bhattacharyya et al., 2016). Though lowland rice paddy significantly affects NEE and NEME, but it has the potential to act as overall net carbon (C) sink (Bhattacharyya et al. 2014).

The GHGs emissions from agriculture, forestry and other land uses (AFOLU) in the Fifth Assessment Report (AR5) published by the Intergovernmental Panel on Climate Change (IPCC) addressed major geophysical and biogeochemical changes, feedback effects and strategies for adaptation and mitigation (IPCC, 2014). Policy focus shifted towards evaluating climate targets. In this connection, different Representative Concentration Pathways (RCPs) of GHG concentrations were discussed in AR5 (IPCC, 2014). Four pathways were identified for climate modelling research. Primarily based on how much GHGs would be emitted in the coming years, RCPs narrated different predicted climatic scenario for future. The four RCPs viz. RCP2.6, RCP4.5, RCP6 and RCP8.5 were characterised depending on radiative forcing values in the year 2100 relative to pre-industrial values (+2.6 +4.5 +6.0 and +8.5Wm⁻², respectively). The RCPs, consistent with wide range of probable changes in future anthropogenic GHGs emissions, aim to represent their atmospheric concentrations. The RCP 2.6 assumes global annual GHG emissions peak during 2010-2020 and substantial decline in emissions thereafter. In RCP 4.5

and RCP 6, emissions peaks would be found around 2040 and 2080, respectively and then declining trends would be expected. However, in RCP 8.5 scenario emissions continue to rise throughout the 21st century.

High temperature adversely affects plants and causes severe cellular injury. This is due to catastrophic collapse of cellular organization and might result to cell death (Schoffl *et al.* 1999). It has been reported that each degree centigrade increase in average growing season temperature might reduce crop yields up to 17% (Lobell and Asner, 2003). According to a study conducted by Porter (2005), increase in temperature coupled with excessive anthropogenic GHGs emissions might lead to shift in geographical distribution and growing season of agricultural crops by altering the threshold temperature for the start of the season and crop maturity. Plants may experience injuries by temperature stress leading to inhibition of growth (Howarth, 2005). Under changing climatic scenario, the response of elevated CO_2 is expected to be higher on C_3 plants (rice, wheat and soybeans), accounting for more than 95% of world's species, than on C_4 plants (corn and sorghum). However, C_3 weeds have also found to respond well to elevated CO_2 levels showing potential for increased weed pressure and reduced crop yields.

Rice is the staple food for majority of the world population. About 90% of the total rice produced is grown in Asia (Bhattacharyya *et al.* 2012). It is grown in different environments under varied climatic (tropical to temperate) and edaphic conditions (Bhattacharyya *et al.*, 2014). Many of the factors controlling gas exchanges between rice paddies and atmosphere are different from dry land agriculture. However, rice production system is considered as a unique anthropogenic manipulator of ecosystem C dynamics via uptake, fixation, emission and transfer of C in soil-plant-atmospheric continuum (Bhattacharyya *et al.*, 2016).

The eddy covariance (EC) technique is widely employed as the most advanced micrometeorological method for monitoring NEE and NEME (Bhattacharyya *et al.*, 2014). The EC technique is one of the most precise methods for measuring trace gas (CO₂ and CH₄) flux exchanges between terrestrial ecosystems and the atmosphere (Swain *et al.*, 2016, 2018). It represents a large area of land typically at the ecosystem scale. It is employed for continental carbon balance from hourly to inter-annual timescales encompassing diurnal variations (Baldocchi, 2003). The EC technique is used for high frequency monitoring of NEE and NEME throughout the crop growing season. It can be coupled with different sensors for ancillary measurements based on research interest (Bhattacharyya *et al.*, 2012; Bhattacharyya *et al.*, 2014).

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2. Estimation of Net Ecosystem CO_2 and CH_4 Exchanges by Eddy Covariance System

In order to quantify global C sink-source budget the EC systems are widely employed to estimate NEE and NEME (Baldocchi et al., 2001). Major components of a typical EC system for conducting research on NEE and NEME include:(i) a sonic anemometer to measure three-dimensional wind speed and sonic temperature, (ii) an open path/closed path infrared gas analyzer to measure the fluctuations in CO₂ and water vapour densities, and (iii) open path CH₄ analyzer to measure CH₄ mole fractions (Fig 1 and 2). Sonic anemometer measures three-dimensional wind speed in real time with 10 Hz friquency. The infrared gas analyzer is a high performance CO₂ and water vapour (H2O) analyzer measuring CO₂ and H2O concentrations in high speed (10 Hz) on real time basis. Likewise, the open path CH₄ analyzer is a fast processing CH₄ sampler operating at 10 Hz scale. These sensors give synchronized output in a fast processing data logger (Bhattacharyya et al., 2014; Swain et al., 2016, 2018). The sonic anemometer, infrared gas analyzer and CH₄ analyzer could be typically installed at 1.5-3.0 m height in a rice field depending on the crop canopy and requirement of the research interest. Infrared analyzer is set back from the sonic anemometer and CH₄ analyzer to avoid obstacles for the approaching eddies coming towards the sensors from the representative fetch area located upwind direction. The sensors viz. anemometer, infrared analyzer and CH₄ analyzer are placed in such way directing towards the prevalent wind direction (Bhattacharyya et al., 2014; Bhattacharyya et al., 2016). In a typical EC system several other ancillary sensors could be fitted for monitoring of different environmental parameters.



Fig. 1 A typical eddy covariance system installed in tropical lowland flooded rice field at the experimental farm of ICAR-National Rice Research Institute, India.



Fig. 2 Sensors of eddy covariance system

3. Eddy Covariance Data Processing and Interpretation

Major assumptions of EC data analyses are as follows: (i)fluxes to be measured only at fetch area, (ii) measurements to be performed inside the atmospheric boundary layer, (iii) horizontal and uniform terrain in fetch area assuming zero average fluctuations, negligible density fluctuations and negligible flow convergence and divergence, (iv) measurements at a point representing fluxes from upwind area, (v) ensuring turbulent eddies and net vertical transfer by eddies, and (vi) precise sensors and instruments to detect very small changes of scalar at high frequency (Bhattacharyya et al., 2014, 2016). However, using customized flux computation applications processing of eddy covariance data could be accomplished. Flux calculations include time series data corrections for errors, gap filling, and computing fluxes on half-hour averaging intervals. Researchers remain vigilant to arrest introduction of probable flux measurement errors in data calculation due to assumptions, problems in instrument, physical phenomena and terrain specificity. Errors in flux measurements are mostly introduced through time response, sensor separation, scalar path averaging, tube attenuation high pass filtering, low pass filtering, sensor response mismatch, digital sampling, sensor time delay, spike and noise, unleveled instrumentation, density fluctuation, sonic heat flux error, etc. A number of corrections are generally performed before processing and analyzing the data to minimize and get rid of such errors.

3.1. Quality Control and Gap Filling of Eddy Covariance Data

Introduction of time lag is essential to address the time lapse required to draw air from the sampling point to the analyzer. By maximizing the correlation between vertical wind speed and concentrations of scalar of interest (viz. CO_{γ}/ CH_4) time



lag is determined (Goulden *et al.*, 996). As described by Aubinet et al (2000) using empirical transfer functions frequency response losses can be corrected. Time series data can be processed on real time basis using customized softwares (viz. EdiRe by University of Edinburgh, EddyPro by LI-COR Biosciences etc.).Coordinate rotation (Kaimal and Finnigan, 1994; Tanner and Thurtell, 1969; Wilczak *et al.*, 2001) and Webb corrections (Webb *et al.*, 1980) are essential during data processing. High spikes in data due to inappropriate meteorological conditions should be eliminated by quality checks. The friction velocity (U*) filtering (Reichstein *et al.* 2005; Papale *et al.*, 2006) for spike detection (Vickers and Mahrt, 1996) are essential for the data sets. The U* is determined on the basis of night time lower threshold limit of velocity of air below which ecosystem gaseous fluxes are ignored. For seasonal NEE gap-filling of missing and/or discarded data, 'look-up' table approach by using empirical relationships among environmental variables is helpful (Falge *et al.*, 2001). However, other gap filling proceduresare also available and can be performed based on user preference.

3.2. Estimation of NEE

Eddy covariance technique is able to precisely assess NEE at ecosystem scale and this has made possible its wide application for determining NEE (Baldocchi, 2003). It can measure NEE across timescales and the footprint. The NEE is generally calculated to sum up the eddy CO_2 flux (flux of CO_2 , Fc) and CO_2 storage change (Fs). However, the Fs can be neglected for NEE calculation as rice canopy height remains relatively low. The mean vertical flux density of CO_2 is calculated by the following formula as proposed by Baldocchi (2003):

 $Fc = \rho a \Box C'$ ------ (Equation 1)

Where, ω' is the 30 min covariance between vertical fluctuations of wind speed; C' is the CO₂ mixing ratio; ρa denotes to air density; time averaging is denoted by over bar; and the primes present indicate fluctuations from average value. A positive covariance indicates net CO₂ transfer to the atmosphere and a negative value designates net CO₂ assimilation by the vegetation. Mean of the 30 min NEE is used to compute daily, monthly, seasonal, annual and inter-annual net exchanges (Bhattacharyya *et al.*, 2014, 2016).

4. Diurnal, Seasonal and Annual Variations of NEE: Case Studies

In tropical lowland flooded rice ecology at the experimental farm of ICAR-NRRI (Cuttack, Odisha, India) daily mean NEE in the crop season showed distinguished

pattern in 2012-13 (Fig. 3). Vegetation phenological stages, temperature fluctuation, soil moisture fluctuations, vegetation canopy structure and variations in light intensity acted as drivers of NEE (Monson *et al.* 2002). Even heat stress, canopy irradiance, stomatal responses, leaf area index and biomass influenced NEE (Patel *et al.* 2011; Nair *et al.* 2011). The NEE throughout the growing season depended significantly on leaf area index (LAI) (Saito *et al.* 2005). Daily variation in net ecosystem productivity (NEP, NEP=-NEE) increased with LAI. The NEP reached its peak around the heading to flowering stage of the crop, thereafter decreased gradually till maturity which could be attributed to the leaf senescence or reduction in leaf greenness during maturity (Pakoktom *et al.*, 2009). The NEE reached its peak at panicle initiation to reproductive stage due to greater physiological activity of rice. Later on NEE became lower at grain filling to harvesting stages (Campbell *et al.* 2001; Alberto *et al.* 2012; Bhattacharyya *et al.* 2013)(Fig 3).

The diurnal variations existed both in CO_2 concentrations and NEE also within the year and or crop growing season (Fig 4, 5). Positive NEE trends were observed from evening through midnight to early morning hours. While, negative NEE were observed mostly at the day time. Plant respiration at night hours led to net efflux of CO_2 to the atmosphere (Fig 5). In daytime atmospheric CO_2 was used up for synthesis by vegetation. In parallel CO_2 was emitted by the soil-vegetation through respiration (Bhattacharyya et al 2013). Negative NEE trend indicated uptake of CO_2 by the lowland rice, whereas, the positive NEE indicated emission from the soil-vegetation continuum.



Fig. 4 Diurnal Variation in CO₂ Concentration Through out the Crop Growing Season

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Fig 3: Annual Daily mean NEE throughout the crop growing season including two fallow periods in 2012-13

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Fig. 5 Diurnal variations in mean NEE in initial (A) and panicle initiation stages (B)of crop growth

The PI and MT stages showed more negative trend in NEE as compared to initial and harvesting stages. This might be attributed to greater greenness in leaves and higher LAI in the wet season as compared to dry season.

5. Estimation of NEME

Methane is recognized as the agriculturally important GHG. The NEME considers net CH_4 exchange between terrestrial ecosystem and atmosphere. Methane emissions from rice paddies at the global scale are being estimated since long time (Bhattacharyya *et al.*, 2016). The mechanism of CH_4 production in anoxic



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soil and its subsequent transport pathways from soil to atmosphere needs to be understood in order to understand CH_4 exchange at atmosphere-rice interface. It has now become possible to measure real time continuous NEME on daily, monthly, seasonal, annual and inter-annual scales by the development of an open path CH_4 analyzer. Moreover, a low power, fast sampling, high precision, self-cleaning CH_4 analyzer facilitates estimation of NEME when fitted with eddy covariance. It has made possible widespread CH_4 flux measurements, needed to understand sourcesink characteristics of CH_4 in various ecosystems.

6. Diurnal, Seasonal and Annual Variations of NEME: Case Studies

The NEME were measured on annual scale using the open path CH_4 analyzer coupled with EC system in tropical lowland flooded rice ecology in 2012-13 at the experimental farm of ICAR-NRRI (Cuttack, India) (Bhattacharyya *et al.*, 2014) (Fig 6).



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Daily mean NEME ranged from around -2 to 10 mg CH_4 m-2 h-1 on annual scale including dry and wet crop seasons and two fallow periods (Fig.6). Higher NEME were found at maxium tillering-panicle initiation and transplanting-maximum tillering stages in both the seasons, respectively. This could be attributed to greater soil labile C, higher physiological and biological activity in plants and rhizosphere zones (Miyata *et al.*2000; Tseng *et al.* 2010; Meijide *et al.* 2011; Bhattacharyya *et al.* 2014). Incoming solar radiation, PAR and net radiation had significant influence on NEME by regulating either stomatal opening or convective flow.Convective flow of CH_4 through plants were regulated by apparent pressure gradient in different plant parts (Hirata *et al.* 2007).

Diurnal NEME (Fig. 7) varied widely in tropical lowland flooded rice ecology at ICAR-NRRI(Fig 8). Peaks in NEME were noticed in afternoon hours. The NEME were higher and distinct in afternoon as also noticed in different parts of the world (Miyata *et al.*2000; Tseng *et al.* 2010). The diurnal variation in NEME could be attributed to the aerenchyma opening pattern of rice in different hours of the day with temperature fluctuations (Schütz *et al.* 1990, Bhattacharyya *et al.*, 2018). Orientation of aerenchymal tissue in rice was found to be major passage/pathway for CH₄ transport of from soil to the atmosphere (Bhattacharyya *et al.*, 2018). Reduction in solar radiation might be responsible for decrease in NEME (Sass and Cicerone, 2002). Relation between leaf biomass temperature and solar radiation could have regulated NEME (Keppler *et al.*2006; Roy *et al.* 2015). Pressure gradients driven by diurnal variation in light availability might have created shifts in NEME. Moreover, dissolved CH₄ in soil solution might have released to the atmosphere via transpiration flux (Nisbet *et al.*, 2009).



Fig. 7 Diurnal mean NEME observed in crop growing seasons

7. Partitioning of NEE into GPP and RE

The NEE estimated by eddy covariance system can be used further for deriving



Gross Primary Production (GPP)and Ecosystem Respiration (RE). Partitioning of NEE into GPP and RE can be accomplished by different empirical models, either by establishing relationship between NEE with temperature or NEE with photosynthetically active radiation (PAR). Models to partition NEE into RE and GPP have several advantages and flaws (Desai *et al.* 2008). Two such models namely, Q10 model and rectangular hyperbolic model are mostly employed for partitioning of NEE. The Q10 model uses relation between air temperature and night time flux to derive RE, whereas, the rectangular hyperbolic model depends on relation between NEE and photosynthetically active radiation (PAR).

8. Net Ecosystem Carbon Balance in Tropical Lowland Rice

Eddy flux derived net ecosystem production (NEP, NEP=-NEE) is considered ideal for C balance in flooded rice ecology (Bhattacharyya *et al.*, 2014). Annual C balance depends on two components, namely, (a) positive component attributing C influx into the system (addition of matter to the rice system) and (b) negative component attributing C out flux from the system (removal of matter from the rice system).



Annual GWP based C budget of Lowland rice ecology (Mg C ha-1)

(Source : Adopted from Bhattacharyya et al., 2013, 2014)

Fig. 8 Schematic diagram of the seasonal carbon balance of lowland flooded rice ecosystem (Mg C ha⁻¹)



Possible measurable C inputs and output components are considered for estimation of seasonal and/or annual C balance in submerged rice ecosystem. As proposed by Smith *et al.* (2010), net ecosystem C balance (NECB) of the rice ecosystem is estimated by the difference between the carbon input-output components using the equation as follows:

NECB = NEP - D - F - H - VOC - NEME - N - E + I ------(Equation 2)

Where, NECB refers to Net Ecosystem carbon balance, NEP is the net ecosystem production (measured by EC system), D is the C loss as dissolved organic C (DOC), F indicates the C loss by fire, H indicates the C loss by harvest, VOC denotes the C loss by volatile organic compound, NEME indicates the C loss as microbially produced CH_4 (converting to GWP equivalent CO_2 -C), E is the C loss by erosion and eluviations and I indicates the addition of C from organic manure and other sources.

9. Case Studies of Net Ecosystem Carbon Balance in Tropical Lowland Rice

Several farm practices and crop biochemical and physiological processes regulated the stored C release and loss from the system. Harvest of above ground crop biomass and grain, DOC loss, net GWP-based CH_4 emissions (expressed in CO_2 -C eq.), erosion, leaching and eluviations encompassed ecosystem C removal. Gaseous C loss due to residue biomass burning and VOC losses were considered to be negligible. The cumulative C losses in terms of the above mentioned processes from the system were 5.02 Mg C ha-1(Fig 8). Lowland rice ecology behaved as net C sink on seasonal and annual basis considering the total C inputs and outputs. Hence, the system had the potential to store C in the order 0.59 Mg C ha–1 y-1. The net C gain was not reflected in soil C build up status. It was assumed that the gained C, might have partitioned into different forms or moved out of the system either in lower soil profiles or converted into certain not easily measured labile C pools (Smith *et al.*, 2010).

10. Conclusion

Soil and water management, agronomic practices, cultivation methods and other agricultural operations influence NEE and NEME during rice cultivation. The EC technique based continuous real time monitoring NEE and NEME help in quantification and validation of GHG flux dynamics in major rice ecosystems under different agro-climatic zones and management practices. This will be helpful in preparing major rice ecology based GHGs emission inventory database preparation and documentation. Moreover, it will also be employed to explore better understanding of trends of GHGs exchanges, which can be further extrapolated to upscale under anticipated climatic changes. As a final outcome this will prove to



be helpful in preparing judicious land use and appropriate management practices to abate GHGs emissions in major rice ecologies by adopting adaptation and mitigation approaches.

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Rice Grain Quality-Issues Related to Climate Change

Srigopal Sharma

1. Introduction

Having achieved self sufficiency in production, the rice development programs now focus on sustaining the yield and enhancing nutritional quality of grain. At present, thousands of rice varieties and land races are grown globally. Proper water and nutrient management practices and pest control measures add not only to grain yield but also to grain quality. Environmental factors such as drought, salinity and temperature extremes impose water deficit stress and impose limits on plant productivity and quality of the produce. Thus, in view of the changing climatic scenario, emphasis needs to be given to understand the impact of global warming and rising levels of greenhouse gases on rice grain quality. High night temperature has been reported to decrease head rice ratio, increase chalkiness, and reduce grain width in rice (Shi et al. 2016). Rice chalkiness, a complex polygenic trait is highly influenced by environmental conditions / cultural practices, particularly during the grain filling stage (Liu et al. 2010; Siebenmorgen et al. 2013). Poor grain quality caused by an increase in night temperature may lead to extensive reduction in economic benefits. Hence, rice grain quality is a complex issue and needs to be understood well, before we deliberate upon the effect of changing climate and environment on this important aspect.

2. Concept of Rice Grain Quality

Rice grain quality varies with the consumer preference and the purpose (end use). The term rice grain quality refers to the visual (physical) characteristics and chemical composition, which decide the marketing, cooking, eating and the nutritional quality. Normally, the grain size, shape and appearance, milling and cooking characteristics

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are the main determinants of quality. Nutritional quality is also an integral part of rice grain quality, which is basically a varietal trait and thus depends on its genetic constitution and is significantly affected by cultural practices, environment and post harvest storing. It is the consumer who decides what kind of rice he/she wants and as taste/liking differs from place to place, the job of the rice scientist/developers is to translate the consumer preference for quality into measurable physical and chemical parameters which can be ultimately traced to a particular gene(s). Grain quality characteristics assume much more importance for rice compared to other food grains and are the prime determinants of market price, because most of the rice (almost 95%) is consumed as cooked whole grain.

2.1. Features of Quality Rice

Quality means different to different people depending on their eating preferences and specific requirement. However, medium /long slender and translucent grains with high head rice recovery (HRR), good cooking and eating quality (good elongating ability during cooking, tender, well separated grains, good mouth feel) and pleasant aroma are normally preferred. The desirable features in quality rice normally include right size and shape, translucency, absence of chalk and cracks, high HRR%, excellent cooking properties (well separated grains, soft texture), good elongation ratio (ratio of lengths of cooked and uncooked grain), no color and good aroma (in case of scented rice).

The rice crop is harvested as paddy or rough rice at 20-24% grain moisture with the mature rice grain (caryopsis) enclosed within an inedible cover called hull or husk. Paddy grains are dried to a moisture content of 14% before processing for quality analysis and to 12%, if the grains are to be preserved for seed purpose. It takes about 3-4 months (ageing period) for the grain quality characters to stabilize, hence paddy grains are analyzed for quality parameters after at least 3 months of harvest.

2.2. Physical Characteristics: Grain Size and Shape

Rice is consumed mainly as cooked whole grain; so size and shape become important and which can be effectively measured with an image analyzer. These are important criteria of rice quality to develop new varieties and also for trade. Different countries have their own classification for rice grain quality. However, in India, Ramiah's classification (Govindaswami, 1985) is still followed to physically categorize grains (Table 1.):

Grain type	Milled Grain Length (mm)	Length :breadth ratio
Long slender (LS)	$\geq 6 mm$	≥3
Short slender (SS)	< 6 mm	≥3

Table 1 Rice grain classification (physical) followed in India

Medium slender (MS)	< 6 mm	2.5 to 3.0	
Long bold (LB)	$\geq 6 mm$	< 3	
Short bold (SB)	< 6 mm	< 2.5	

2.2.1. Chalk and cracks

Grains with opaque areas (white belly, white centre, white back) in the endosperm caused due to loose packing of single starch granules and protein molecules is an undesirable trait (although Italians like it) and referred to as the chalky grains; such grains show more breakage on dehulling and milling compared to translucent grains and absorb more water (due to air spaces) during cooking resulting in soft cooked rice. Grain chalkiness is greatly affected by environmental conditions during cultivation. In some seasons, cracking of the rice grain is a major problem. Most cracking occurs in the field and seems to be related to changes in grain moisture or to moisture cycles after the rice matures. Cracking may also result from rain on dry grain and storage of grain with variable moisture levels. It decreases HRR, because cracked grains often break during milling and fetch low payments by the grower and the miller. Cracks also decrease the cooking quality of the grain. Rough handling of grain during harvest operations, drying and processing also cause the grain to crack. The marketable quality depends on appearance (length, breadth, HRR, chalkiness, color, dockage and packaging).

2.3. Milling Quality

The paddy is made free from immature grains, dockage and brought to 14% moisture level before further processing. It is then dehulled through rubber rollers to minimize breakage of grains to obtain 'brown rice' which has a colored (pink, brown, red blue, black) coating that is rich in vitamins, minerals, oil and other nutrients and is thus prone to infestation by insects and microbes. When the brown rice is passed through an abrasive whitening machine the colored coat is removed as brownish powder called bran. And white rice grains is called milled rice. It is further passed through a friction type whitening machine resulting into a smooth final product called polished rice, which we are used to eat. Milling is done to remove bran and germ with minimum breakage of whole grain. It is produced commercially by millers because it has longer shelf life and a better appearance than the brown rice. However, brown rice has latter nutritive value than polished rice. Thus, normally, it is the milled rice characteristics we refer to while describing the grain quality. Milled rice kernel with 75% or more of the average length of the whole kernel is called head rice. Percentage of head rice recovered during milling of paddy is called milling yield or head rice recovery (HRR). High milling yield (> 60%) is the first condition for a variety to be successful. For long slender grains it may be between 55-60%. The sum total of the amounts of head rice and broken rice

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obtained from a paddy sample is called milling recovery which is generally about 70%. The millers prefer rice with high percentage of hulling, milling and HRR. The HRR is the single most important factor that determines market price of rice as it is normally eaten as whole grain.

2.4. Cooking Quality

A very important indicator of good cooking quality of rice is that the cooked grain retains a firm shape and does not disintegrate during or after cooking. Varieties that do not meet this requirement are not commercially successful. Starch forms the major part (about 90%) of rice kernel. Hence, the cooking quality is mainly governed by the packaging of starch molecules and the amylase : amylopectin ratio. The cooking quality is determined by alkali spreading value (ASV), gelatinization temperature (GT), water uptake (WU) value, volume expansion ratio (VER), kernel length after cooking (KLAC), elongation ratio (ER), gel consistency (GC) and apparent amylase % (AC%).

Alkali spreading value (ASV) is measured by treating six rice grains in a Petri plate with 10 ml of 1.7% KOH for 23 hours at 300C and looking for disintegration of grains, on a 1-7 scale. Gelatinization temperature, (GT, the temperature at which the starch granules swell in water irreversibly losing their crystallinity) is indicated by alkali digestion. It ranges from 55-790C. GT is high for high amylose rice, but low amylose rices have also been found to have high GT. The waxy or low amylose rices have more free sugars and maltodextrins giving it sweetness. The differential scanning calorimetry (DSC) gives the accurate measure of GT rather than ASV. These two are indicators of digestibility of a rice grain. Water uptake value (WUV) is measured in terms of the volume of water absorbed by 100g of grains at 80oC . VER (volume expansion ratio) is a measure of the increase in volume of rice after cooking. KLAC and ER (mean length of ten cooked rice grains divided by mean length of the raw milled grains) measure lengthwise elongation during cooking. Gel consistency (GC) measures the tendency of cooked rice to harden on cooling, especially for high amylose rice. Rice with soft GC cook tender and remain soft even upon cooling and hence is preferred by consumers and is a priority for breeding programme. The cooking and eating quality is determined mainly by amylose content. Hence, all rice improvement programs target apparent amylose content as a parameter. It is normally measured by the iodine binding capacity (IBC), though other methods like near infrared (NIR) grain analyzer, size exclusion chromatography (SEC) and Nuclear Magnetic Resonance (NMR) are also used.

2.5. Nutritional Quality

Rice is an integral part of the diet of about half of the global population and accounts for 35-75% of the calories consumed by more than three billion Asians (Khush,

2007). Being a cereal crop, it is rich in starch, contains little fat and on average 7% protein of excellent quality. Milled rice contains about 80% carbohydrates, which include mainly sugars and starch. In general, a low ambient temperature during grain filling results in increased amylose content in rice and vice-versa. The high amylose rice shows high volume expansion and flakiness. The cooked grains are dry, less tender and become hard upon cooling, whereas, the low amylase-rice cooks soft and sticky. The intermediate amylose rice is normally preferred world over, except the places where Oryza japonica is liked. Milled rice normally contains about 7% total protein though some germplasm contain up to 16% protein. The brown rice contains up to 2.8% lipids. Milled rice has 0.64% lipid and the rice bran contains 19% lipids from which oil is extracted. Brown rice is richer in minerals compared to milled rice. Milling reduces percentage of P (0.28 to 0.06), K (0.21 to 0.05), Mg (0.10 to 0.015), Ca (0.013 to 0.008), Mn (17.7 to 5 ppm), Fe (12 to 5 ppm) Zn (27 to 16 ppm) and Cu (3 to 2.5 ppm) (Hunt et al. 2002). Brown rice has more amounts of vitamins than the milled rice because they are present mainly in bran. Most of them are lost to different degrees during milling and subsequent washing. Rice is a good source of vitamin E (tocopherols) and tocotrienols. The vitamins A, C and D are not present in rice. Basmati rice and the small or medium grain non-basmati rice have pleasant aroma and are classified under quality rice. The aroma of scented rice is mainly due to 2-acetyl 1-pyrroline (2-AP). Basmati rice contains about 0.09 ppm of 2-AP which is about 12 times more than that present in non-aromatic rice. The 2-AP content is measured with GC-MS. For screening, freshly cooked rice is subjected to a sniff test in a test tube or raw rice is digested with dilute alkali. High temperature is most likely to reduce the 2- AP content in scented rice, particularly the basmati rice, as the aroma compound is volatile in nature.

3. Climate Change and Rice Grain Quality

The shadows of two natural disasters namely, climate change and water deficit are looming large on our planet. Rising levels of greenhouse gases and increasing atmospheric temperature have already begun to exert adverse effect on global climate. Carbon dioxide level and temperature are two vital factors whose interaction will greatly determine the overall effect of climate change on agriculture in terms of quality as well as quantity of the produce.

3.1. Climate Change and Atmospheric Carbon Dioxide

Carbon dioxide is essential to plant growth. Atmospheric carbon dioxide (CO_2) has increased about 35% since 1800 from 280 to 400 ppm, in 2013 and computer models predict that it will reach between 530 and 970 ppm by the end of the century (IPCC, 2007, 2014). Currently, its amount in the atmosphere is 407 ppm, compared to oxygen (210,000 ppm). Increased CO_2 is expected to have positive



physiological effects by increasing the rate of photosynthesis. The effect of an increase in CO_2 would be higher on C_3 crops (like rice) than on C_4 crops (such as maize), because the former is more susceptible to carbon dioxide shortage. Thus, rising CO_2 concentration in the atmosphere can have both positive and negative consequences. Though there are some studies made on predicting the effect of climate change on the yield of important crops, but studies on quality aspect are very few. Rice quality is likely to change with elevated CO_2 levels, both alone and with increased temperature. High temperatures during the grain filling period are known to have adverse effect on the rice quality.

3.2. High Temperature Stress on Rice Grain Quality

The global mean surface air temperature increased by about 0.9°C in the 20th century and is projected to increase by 1.4 to 5.8 °C in the 21st century. Extremely high temperature during vegetative growth reduces tiller number and plant height, and negatively affects panicle and pollen development, thereby decreasing rice yield. High temperature is of particular importance during flowering, which typically occurs mid-morning. Exposure of rice crop to temperatures exceeding 35°C for a few hours can greatly increase spikelet sterility by reducing pollen viability and therefore cause irreversible yield loss. Studies conducted at IRRI, Philippines in the early 1980s demonstrated significant genotypic variation in high-temperatureinduced spikelet sterility leading to identification of tolerant varieties.

Another strategy to avoid high-temperature-induced spikelet sterility is to change the time of flowering commencement into the cooler periods of the day to escape high temperature. Wild rice and Oryza glaberrima accessions evaluated at IRRI varied by about 4 to 5 hours in 'flowering timing' in a day. Selecting for earlymorning floret opening could protect rice fertility from future adverse effects of climate change. The development of rice that tolerates or avoids high temperature during flowering is essential for rice production in the future. It will also benefit rice farmers immediately. Grain yield reduction by a 1°C rise in mean daily temperature is about 5-7% for major crops, including rice. This yield reduction is mostly associated with a decrease in sink formation, shortening of growth duration, and increase in maintenance respiration. The annual average minimum temperature increased at 0.04°C per year from 1979 to 2003 at IRRI. This increase in minimum temperature was three times greater than the increase in maximum temperature over the same period. More importantly, rice yield declined by 10% for each 1°C increase in growing-season minimum temperature in the dry season. The effects of increasing minimum temperature on rice growth and yield are less understood. Okada et al (2011) have developed a model that predicts rice-quality response to changes in temperature and radiation levels. Declines in rice quality have been observed in western Japan, since 1990s. The researchers' model suggests

that rice quality will decrease even further if management practices remain the same. The main reason for the decline in rice quality is the occurrence of chalky grains, especially milky-white grains. Chalky grains sharply increase when the mean daily minimum temperature for the 20 days after heading (ear emergence) exceeds 22°C. The underlying mechanisms for the occurrence of chalky grains in rice plants are, reduced carbohydrates in the plant associated with an increased night-time respiration rate; reduced capacity of stems and leaves for assimilation; insufficient solar radiation during the ripening period; and hits of typhoons during the ripening period.

3.3. New Rice Plant Type: To Maintain High Yield and Grain Quality under High Temperature

Increased maintenance respiration results into loss of biomass; differential effects of night vs. day temperature on growth and crop phenology have been proposed as possible causes. There is limited information on genotypic variation of rice respiration in response to increased temperature and management factors. The average daily temperature during grain-filling has a detrimental effect on at least three components of grain quality: chalkiness, amylose content, and gelatinization temperature. High temperature shortens the duration of grain filling, but the rate of grain-filling decreases because a number of the genes/enzymes involved in starch synthesis are sensitive to high temperature. The shorter duration coupled with the slower turnover rate significantly decreases the proportion of well-filled grains and increases the proportion of chalky grains. High temperature causes the gelatinization temperature of rice to increase, which changes the cooking time and the cooked texture of rice. High temperature during grain-filling also changes the amylose content. Amylose contributes, independently or interactively, to every trait of cooked rice Hence, research needs to be undertaken to develop new rice varieties that maintain yield potential and grain quality under high temperature by understanding the genetic, molecular, and physiological mechanisms of fertilization, respiration, spikelet fertility, and grain quality under high temperature. Another area which needs to be studied is to determine the genotypic variation during day when flowering occurs. Information on how far the nitrogen and water management are effective to minimize the negative effects of high temperature on grain yield and grain quality is also needed.

3.4. Chalkiness is a Varietal Characteristic Affected by Environment

The endosperm of waxy rice is opaque but sometimes the endosperm of the commonly eaten non- waxy rice grains also has opaque areas in an otherwise translucent grain; such grains called chalky grains, which break easily during hulling/ milling resulting in poor market price. There were some varieties in the Philippines that were rarely chalky. Their panicles had very few secondary branches, indicating that



panicle architecture is under genetic control and these genes play a role in chalk. It was reported by Resurreccion and Fitzgerald (2007) that high temperature reduces the time for which the panicle serves as sink. The grains on primary branches are of highest priority in the panicle and are translucent, whereas, grains on secondary branches are of lowest priority. As the supply of sugars from vegetative parts to panicle (sink) ceases, the grain filling stops resulting in immature or chalky grains. Therefore, varieties with large panicles and high number of secondary branches (like IR 8) is more likely to form chalky grains when environmental conditions such as high temperature shortens the grain filling period (time for which panicle is sink) compared to those with a small panicle with fewer secondary branches (e.g., IR 60). The issue assumes importance in view of the present trend towards global warming. A single recessives gene pgwc-8 (percent grains with chalkiness-8) is identified which controls chalkiness.

In elevated carbon dioxide, the proportion of grains containing a high amount of chalk per grain will decrease which would further increase the market value of the grain and may help to alleviate the burden of climate change on rice farmers. As environmental conditions affect starch content, the climate change is likely to affect chalk, amylose and GT. The positive effect of high CO₂ are not likely to compensate for negative effects of high temperature on grain quality. A temperature rise of just 20C is sufficient to trigger this trait. Researchers have noted that a 40C increase could ruin entire crops.

Experiments were done with rice plants grown at 260C and 330C. It was found that at the higher temperature, plants had only half as many days in which to make grain (14 compared with 30). Thus, the time devoted to grain production was reduced by high temperature. At one extreme, the plant attempts to fill all grains, resulting in high yields of low-quality, chalky rice. At the other end, the plant sacrifices half the grains, resulting in low yields of high-quality grain. Variation in this stress response was also found to be under genetic control. Thus, scientists suggest minimizing secondary branching in the panicle, extending the time available for grain filling, and selecting for a heat-stress response to avoid chalkiness.

3.5. Climate Change and Agronomic/Physiological Parameters

Researchers at the Tamil Nadu Agricultural University, Coimbatore conducted a study on rice which involved actual cultivation of the cereal in a climate control chamber where the temperature was maintained at four degrees above the ambient temperature and a carbon dioxide enrichment level of 650 ppm. The experiment was carried out with four different days of planting: June 1, June 15, July 1 and July 15. It was seen that crops grown under the projected conditions attained panicle initiation, flowering and maturity much earlier than those grown under the ambient condition. But, recorded reduced growth characters such as leaf area index, dry

matter production and number of tillers. In addition, lesser percentage of dry matter was partitioned towards grain and more for the roots. Subsequently, they recorded lower grain and straw yields. Overall, elevated temperature was found to have a negative impact on rice productivity, even nullifying the positive effects of higher level of carbon dioxide.

3.5.1. Climate Change and Amylose Content, Gel Consistency and GT

Amylose content of rice grain, a major determinant of cooking quality is known to increase under elevated CO₂ conditions. Thus, cooked rice grain from plants grown in high-CO₂ environments in future would be firmer than that from plants grown today. However, when the quality traits of varieties grown in four combinations of temperature and carbon dioxide levels were assessed (Zhong et al. 2009), the negative impact of temperature on grain quality was not overcome by an increase in CO₂. Four cultivars with different amylose content (AC) were subjected to two temperature treatments, referred as optimum (mean daily air temperature, 22 °C) and high (32 °C) temperature regimes starting from flowering stage until maturity. Effect of high temperature on AC and GC in milled rice was found to be cultivar-dependent. Under high temperature, AC increased for cv. Jiayu353 and remained little changed for cv. Guangluai4, which had intrinsically higher AC, and decreased for cv. Zhefu49 and cv. Jiazao935, which had lower AC. By contrast, high temperature maintained stable GC values for cultivars with higher AC and increased GC values for those with lower AC. Moreover, high temperature significantly increased the GT of all cultivars. Pasting profiles and X-ray diffraction pattern of rice were also affected by temperature. The results suggest that high temperature during grain filling can change the component and crystalline structure of starch and result in deterioration of eating and cooking quality for early-season indica rice.

3.5.2. Climate Change: Protein, Iron, Zinc and B Vitamins Content

Rising atmospheric concentrations of CO_2 could dramatically influence the performance of crops, but experimental results have been highly variable. For example, when C_3 plants are grown under carbon dioxide enrichment, productivity increases dramatically at first. But over time, organic nitrogen in the plants decreases and productivity diminishes in soils where nitrate is an important source of this nutrient. In C_3 plants, elevated carbon dioxide concentrations inhibit photorespiration, which in turn inhibits shoot nitrate assimilation. Thus, agriculture would benefit from the careful management of nitrogen fertilizers, particularly atmospheric carbon dioxide concentrations rise and nitrate assimilation diminishes, these crops will be depleted of organic nitrogen, including protein, and food quality will suffer (Taub *et al.* 2008).

NRRI

A recent study (Zhu et al, 2018) on average reduction in grain protein at elevated (568 to 590 μ mol mol-1) relative to ambient [CO₂] for 18 cultivated rice lines of contrasting genetic backgrounds grown in China and Japan using FACE technology showed that when grown under field conditions a significant reduction (an average of -10.3%) in protein relative to current [CO₂] was observed for all rice cultivars. Similarly, significant reductions in iron (Fe) and zinc (Zn) were also observed (-8.0 and -5.1%, respectively) among all rice cultivars tested. On the basis of [CO₂] assessment per se, there were no significant site difference effects on rice grain quality between Japan and China (P = 0.26, 0.17, and 0.10) for protein, iron, and zinc. There were also declines of 17% in the vitamins B1 (thiamine) and of more than 16% in vitamin B2 (riboflavin). Vitamin B5, or pantothenic acid levels were down more than 12%. Folate or vitamin B9 levels were down 30%.

The studies of Li et al (2018) with 155 RILs showed that the environmental conditions have a stronger effect on protein content and alkali consumption than genotype, while genotype has stronger impact on AC and grain width than environmental factors.

Wheat, rice and potato provide 21%, 14% and 2% of protein, respectively, in human diet (FAOSTAT, 2007). Grain protein in rice (Terao *et al.* 2005) declined by about 10% at elevated carbon dioxide concentrations. Similarly, at elevated carbon dioxide and standard fertilizer levels, wheat had 10% less grain protein (Fangmeier *et al.* 1999; Kimball *et al.* 2001). Several approaches could mitigate these declines in food quality under carbon dioxide enrichment. Increased yields may compensate to some degree for total protein harvested. Several-fold increases in nitrogen fertilization could eliminate declines in food quality (Kimball *et al.* 2001), but such fertilization rates would not be economically or environmentally feasible given the anticipated higher fertilizer prices and stricter regulations on nitrate leaching and nitrous oxide emissions.

Greater reliance on ammonium fertilizers and inhibitors of nitrification (microbial conversion of ammonium to nitrate) might counteract food quality. Nevertheless, the widespread adoption of such practices would require sophisticated management to avoid ammonium toxicity, which occurs when plants absorb more of this compound than they can assimilate into amino acids and free ammonium then accumulates in their tissues (Epstein and Bloom 2005). Several of these issues might be simultaneously addressed by fertigation, or frequent additions of small amounts of ammonium-based fertilizers in water delivered through micro-irrigation. Moreover, the protein content of the grain decreases under combined increases of temperature and CO_2 (Ziska *et al.*, 1997). Studies have shown that higher CO_2 levels lead to reduced plant uptake of nitrogen (and a smaller number showing the same for trace elements such as zinc) resulting in crops with lower nutritional value . However, concentrations of iron and zinc, which are important for human nutrition, would


be lower under high temperature stress (Seneweera and Conroy, 1997). This would primarily impact on populations in poorer countries, less able to compensate by eating more food, more varied diets, or possibly taking supplements.

Studies by Liu et al (2017) indicated that the ecological indices, rice phenology, and leaf area would decrease under a simultaneous increase of CO_2 and temperature. For the physiological indices, Malondialdehyde (MDA) levels increased significantly in the seedling period. However, it showed the trend of increase and subsequent decrease in the heading and filling periods. In addition, the decomposition of soluble protein (SP) and soluble sugar (SS) accelerated in filling period. The rice quality index of the Head Rice Rate showed the decreasing trend and subsequent increase, but the Chalky Rice Rate and Protein Content Indices gradually decreased while the Gel Consistency gradually increased.

Neumann et al, (2017) found that arsenic uptake by rice could increase due to global warming that threatens yield and contaminating grain. The XRF imaging revealed greater arsenic sequestration in root iron plaques with a warmer soil temperature. Mean and median arsenic concentrations in pore water and root, straw, and husk tissue were positively correlated with average daily maximum soil temperature, though the grain arsenic concentrations did not change. This could possibly be attributed to increased reductive dissolution of arsenic bearing iron minerals, but the plants effectively regulated grain arsenic.

3.5.3. Sucrose Content and Sucrose Cleaving Enzyme Activity in Rice Grain during the Filling Stage

In grains of two japonica rice varieties Koshihikari and Sasanishiki, the sucrose synthase activity was higher than that of invertase which was significantly correlated with starch accumulation rate, indicating that the sucrose synthase played an important role in sucrose degradation and starch synthesis. Under high temperature, the significant increase in grain sucrose content without any increase in fructose and glucose contents, suggested that the high temperature treatment enhanced sucrose accumulation, while diminished sucrose degradation in rice grains. (Li Tian *et al.* 2005).

4. Conclusions

The traits of physical quality of grain include length, width, uniformity, weight, head rice yield, color (whiteness and translucence), chalk, and cracks. The cooking and eating characteristics of rice are determined by amylose: amylopectin ratio, gelatinization temperature, viscosity, texture of cooked rice, flavor and aroma. Significant reduction in protein, iron, zinc and some of the vitamins would affect the nutritional quality of rice adversely. Most of these rice grain quality characteristics are likely to be adversely affected by high temperature and global



warming related to climate change. The rise in CO_2 could benefit the C_3 rice, which convert atmospheric CO_2 into carbohydrates and other organic compounds through photosynthesis. The extent of this benefit, however, remains uncertain due to the simultaneous increase in temperature and the complex relationship between carbon and nitrogen metabolism in plants (Finzi *et al.* 2007; Johnson 2006; Reich *et al.* 2006). The results showed that environmental factors had variable effects on rice grain quality, ranging from the heading stage to the mature stage. Based on the vast data, it is suggested that adjusting the sowing date may help mitigate at least partially the current and the expected environmental conditions and improve rice quality. Hence, to ensure rice food security, the future rice breeding programme should focus on developing climate-resilient rice varieties with higher head rice yield and superior grain and nutritional quality.

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ICAR-

Theme: II

Rice-physiology and impacts of climate change on rice-photosynthesis



Photosynthesis, Biomass Allocation and Productivity in Rice

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1. Introduction

Rice yields need to be increased by at least 60%, to provide adequate food and nutrition to the global population that is expected to reach 9 billion by 2050 (FAO 2009). Rice is the staple food of over half of the world's population and the rice consuming population is increasing at the rate of 1.098% per annum. Increasing population means more demand for food, water and land at a time when the natural resource base for agriculture is being degraded because large areas of farmland are being diverted from food production to industrialization and bio-fuel production. Unpredictable climate change is threatening to further reduce agriculturally viable land due to more instances of drought and flood affecting food demand. Therefore, it is essential that we continue to improve crop performance in terms of grain productivity to keep pace with population growth. India has the largest area under rice in the world. Rice is cultivated in 534 districts of the country. Out of which, 218 districts are having productivity below the national average. Thus, 41% of total rice growing districts are above the national average productivity and

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remaining 59% districts are below the national average productivity. It is evident from the above data that major rice growing areas are below the national average productivity. It is, therefore, pertinent to make concerted efforts to increase the productivity of those areas having low and very low productivity in particular and overall productivity in general.

Plant growth correlates with net carbon (C) gain on a whole plant basis (Kruger 2006). Photosynthetic rates vary between species, but leaf nitrogen (N) content and light intensity are known to be major determinants (Wright et al, 2004). The other main factor determining plant carbon gain is leaf area per plant, which is a function of mean leaf area and leaf number. In grasses, the production of secondary stems by tillering can greatly increase leaf number per plant, with tiller numbers appearing to be mediated by plant nutrient status, light availability and competition for light between plants in a stand.

A review (Poorter, 2012) looked at the factors controlling the allocation of biomass to leaves, stems, and roots in a wide range of species. The most important factors were determined to be the quantity of light available (daily photon irradiance), nutrient and water availability, temperature, and plant age/size. However, two of these, water availability and temperature, tended to have large effects only at extremes—very low temperatures, and either strong water deficits or water logging conditions. Thus, for most plants, light intensity and nutrient availability seem to be the primary factors driving carbon acquisition and biomass allocation.

Grasses (family Poaceae) are a plant group of unique importance in agriculture. Grasses contribute more than half the calories consumed worldwide—both directly, as cereals, and as forage grasses that form the basis for the production of meat and milk worldwide (Raven, 2010). Thus, a complete understanding of the factors determining the productivity of grass plants is of fundamental importance in meeting the food needs of the growing human population. Cultivated and semi-natural grasslands cover an area estimated at 52 million km2, and account for approximately 15% of global primary productivity (Rehuel, 2010). Grass architecture is based around a tiller axis comprised of a stack of phytomers with an apical meristem responsible for the production of new phytomers (Fig 1). Each phytomer has the capacity to produce one leaf, one tiller, and one or more roots. The tiller axis of vegetative grasses is generally small, and is often located around ground level, while the leaves grow from the base, as adaptations to prevent grazing damage to the meristems. Perennial ryegrass (Lolium perenne L.) has on average three leaves per tiller, with the initiation of new leaves coinciding with the senescence of the fourth leaf (Fulkerson, 2001), while cereal grasses may have a much higher number of leaves.



Fig. 1 Schematic of a mature phytomer and its components, and arrangement of phytomers along a tiller of C. squarrosa (Adapted from Yang *et al.*, 2016)

Under competitive conditions, we expect plants to pursue a strategy, where they use resources such as N and water to maximize growth and ultimately reproduction. Photoassimilates represent both the substrate for growth, and also energy storage for biochemical activities. The partitioning of these resources can have significant implications for plant productivity. For example, generally in grasses, around 80–85% of plant biomass is partitioned to aboveground organs, such as leaves and stems, with15–20% is allocated to roots. However, root mass allocation can be significantly affected by nutrient or light availability (Poorter, 2012), with greater carbon allocation to the root system under low nutrient, high-light conditions. Even within the root system, the distribution of roots is frequently non-uniform, with localized root proliferation common in nutrient rich patches (Robinson 2007). It is worth stressing that the optimal biomass allocation will depend heavily on environmental constraints.

Plant performance is also strongly associated with, and dependent on, plant development and growth. Several developmental features of plants, such as overall plant architecture, leaf features and vasculature architecture, are major traits that determine the overall performance of crop plants (Fig. 2). Thornley (1972) developed an early model of vegetative plant growth and biomass allocation based upon carbon and nitrogen uptake and assimilation. Thornley's model holds that carbon fixed in the shoot is either used in the shoot or transported to the roots. Similarly, nitrogen absorbed by the roots can either be used directly in situ, or transported to the shoot. In the case of roots, carbon from the stem can be respired to provide energy for metabolic processes such as nutrient uptake and assimilation,



or used in growth to explore the soil for further nutrients. Meanwhile, nitrogen in excess of that required by roots can be transported to the shoots for use there. Thus, in order to understand the partitioning of carbon within plants, we need to understand the factors, which determine the size of the carbon/nitrogen pool and its usage in each organ. While quantitative descriptions of factors driving carbon allocation are becoming increasingly robust (Poorter, 2012), there remains much to be done on understanding the underlying mechanisms in context. This chapter aims to highlight some of these processes.



Fig. 2 Plant developmental features that are relevant to crop biomass and yield. (Adapted from Mathan *et al.*, 2016)

2. Leaf Photosynthetic Parameters Related to Biomass

Natural variations are a major approach to identify new options to improve crop light use efficiency. So far, successes in identifying photosynthetic parameters positively related to crop biomass accumulation through this approach are scarce, possibly due to the earlier emphasis on properties related to leaf instead of canopy photosynthetic efficiency. Improving photosynthetic efficiency is regarded as a major target to improve crop biomass production and yield potential (Long *et al.*, 2006; for review, see Zhu *et al.*, 2010). The canopy photosynthetic efficiency, which is determined



by leaf area index, canopy architecture, and leaf photosynthetic properties, plays an important role in determining biomass accumulation (Long et al., 2006; Zhu et al., 2012). Historically, the improvement of canopy architecture (i.e. creating cultivars with semidwarf architecture, more erect leaves, and higher leaf area index) has played an important role in traditional crop breeding (Hedden, 2003; Peng et al., 2008); in contrast, the improvement of leaf photosynthetic properties has played a minor or no role during this process. Broadly, there are two major approaches to improve photosynthetic efficiency: by genetically engineering photosynthetic efficiency if an engineering target is well defined and by conventional crop breeding i.e. identifying those lines with superior photosynthetic efficiency and then crossing this superior photosynthetic property into desired target cultivars (Gepts, 2002; Long et al., 2006). In either case, the major challenge now is to define effective photosynthetic traits that can lead to enhanced biomass production. A systems approach can be used to identify new targets to improve photosynthesis by combining systems modeling and an evolutionary algorithm (Zhu et al., 2008). The identified targets to improve photosynthesis have been tested transgenically both in the laboratory and in the field (Rosenthal et al., 2011; Simkin et al., 2015). Similarly, increasing the speed of recovery from photoprotection has been demonstrated to be a major approach to increase canopy photosynthesis and crop yield potential (Zhu et al., 2004), which was validated recently in the model crop species tobacco (Nicotiana tabacum) in the field (Kromdijk et al., 2016). This success of enhancing biomass production through the manipulation of photosynthesis clearly demonstrates that there is huge potential to improve photosynthetic efficiency for greater biomass and yield production. So far, however, large-scale systematic studies of natural variations of photosynthetic parameters in major crops are scarce. Driever et al. (2014) reported natural variations of photosynthetic parameters in 64 elite wheat (Triticum aestivum) cultivars and found that, although there are significant variations in photosynthetic capacity, biomass, and yield, no correlation exists between grain yield and photosynthetic capacity. They suggested that, during the breeding process, some traits might have been unintentionally selected out; hence, photosynthetic efficiency should be a major target to utilize during wheat breeding in future (Driever et al., 2014; Carmo-Silva et al., 2017). Similar experiments have been conducted in rice that reached similar conclusions (i.e. leaf photosynthetic rates measured under saturating light levels do not show positive correlation with biomass accumulation).

Further, improvement in Harvest Index (HI) is difficult to achieve. Peng *et al.* (1999) suggested that further improvement in rice yield potential might come from increased biomass production rather than increased HI. However, we hypothesize that HI is more important in determining grain yield than biomass production under sub-optimum and unfavorable growing conditions even when comparison is made among the modern rice cultivars. At first sight, this is rather contradictory to the current theory of photosynthesis. However, if we consider the canopy, then



the overall crop light use efficiency, where biomass accumulation can be used as a surrogate, is determined by the total canopy photosynthesis instead of leaf photosynthesis. Indeed, earlier work showed that light-limited photosynthesis can contribute up to 70% of the total canopy photosynthetic CO_2 uptake rates, even at a moderate leaf area index of 4.8 (Song *et al.*, 2013). The proportion of light-limited photosynthesis will be even higher under either high leaf area index or future elevated CO_2 conditions (Zhu *et al.*, 2012; Song *et al.*, 2013). Large-scale surveys of rice grain yield, harvest index, and biomass accumulation for rice cultivars released since 1966 have clearly shown that the grain yield of cultivars released after 1980 was highly correlated with biomass accumulation, suggesting improved canopy photosynthesis during recent rice breeding (Peng *et al.*, 2001; Hubbart *et al.*, 2007). However, the potential factors contributing to canopy photosynthesis in rice remain unknown.

3. Photosynthetic Carbon Assimilation and Fixation

Given the priority of photosynthesis in plant growth, it seems logical to first discuss factors influencing photosynthetic rate. However, given the size and complexity of the subject area, this is not intended to be a comprehensive analysis of photosynthesis, or of the factors controlling photosynthetic rates. Rather, the goal is to briefly explore a few of the more important factors, first on an individual plant basis, and then for competing plants later. Breeders have managed to increase yields via processes that alter carbon partitioning rather than improving photosynthesis (Fischer *et al.*, 2014). Breeding better crops through improved photosynthesis is along-sought goal but so far has remained unrealized because of the multiplicity of challenges involved (Evans, 2013). Different options that are being studied to improve photosynthesis in crop plants are summarized below (Fig. 3).



Fig. 3 A number of options that are being studied to improve photosynthesis in crop plants (Adapted from Douglas *et al.*, 2018).

At a biochemical level, the inefficiencies of C_3 photosynthesis are well understood, and mainly depends on the enzyme Rubisco (Yamori, 2016). The CO₂ fixing enzyme, ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco; EC₄.1.1.39), has a very low catalytic efficiency; thus requiring the investment of 20–30% of leaf nitrogen in Rubisco (Gletch 2014. Given its inefficiency, and the relatively high proportion of leaf nitrogen for which Rubisco accounts, a strong positive correlation between photosynthetic rates and leaf nitrogen concentration has been noted (Evans,1989; Makino 1997]. As we know, this requirement for high concentrations of Rubisco in photosynthesizing leaves limits productivity, and has profound implications for plant form and productivity. Photorespiration is the process by which Rubisco fixes oxygen rather than CO₂, with the resulting production of CO₂ and ammonia, and represents a second major inefficiency of C₃ photosynthesis—and thus plant productivity—with up to one-third of light energy intercepted by the plant used in photorespiration.

Photosynthesis and photorespiration are competitive, with an increase in CO₂ or a decrease in O₂ having equivalent effects of increasing carbon fixation. Indeed, classical studies show that growing Phaseolus vulgaris L. at reduced oxygen levels led to a 40% increase in photosynthetic CO₂ uptake (Parkinson, 1974), while a 30% increase in grain yield was noted in rice plants grown at elevated CO₂(Yoshida, 1973). Thus, it is generally assumed that increasing atmospheric CO_2 levels will likewise lead to a decrease in photorespiration, and an increase in plant productivity. However, recent evidence suggests that malate formed during photorespiration is an important source of energy for the reduction of nitrate to nitrite—the first step in nitrate assimilation. Free air carbon dioxide enrichment (FACE) experiments suggest that future increases in atmospheric CO₂ levels may suppress photorespiration, leading to grain protein levels between 7% and 10% lower than current conditions in C_3 plants—with negative consequences for human nutrition and health globally. C_4 plants use a biochemical pump to increase the CO₂ concentration around Rubisco, thus suppressing photorespiration. C4 Rubisco has carboxylation rates up to double those of C₃ plants (Bloom, 2012) with C₄ plants achieving superior photosynthetic nitrogen use efficiencies (CO, fixed per unit nitrogen) due to their reduced N requirement for photosynthesis. Maize (Zea mays L.) and sorghum (Sorghum bicolor L.) are agronomically important C_4 grasses, with both species used both for grain and as silages for animal fodder. However, given that approximately 46% of the estimated 10,000 grass species use C_4 photosynthesis (Sage, 2015), there must be many other C_{4} grasses which would be suitable targets for improvement for forage, despite their generally low nutritive value. The C4 photosynthesis has evolved atleast 24 times in the grasses (Studer, 2014), and there are significant efforts currently to engineer C4 rice with the goal of significant increases in grain yield (Von Caemmerer, 2012).

Inorder to boost food production for a rapidly growing global population, crop yields must be significantly increased. One of the avenues being recently explored is the improvement of photosynthetic capacity by installing the C₄ photosynthetic pathway into C3 crops like rice to drastically increase their yield. A further benefit of developing C_4 agricultural species might be an improvement in stress tolerance. In sorghum, drought induced yield declines in plants grown under elevated CO, conditions, suggesting that future increases in atmospheric CO₂ conditions may alleviate drought stress, even in C4 plants (Conley, 2008). The catalytic rates of Cyanobacterial Rubisco has approximately four-times those of C₂ plants, and might drive up carbon fixation rates and growth, or atleast reduce N requirements for photosynthesis. This might be useful in crops destined for biofuel production, but the low tissue N levels would provide poor nutritive ability for animals. Rubisco overexpressing (125% of WT) rice plants exhibited 32% and 15% increased biomass when grown at 280 and 400 ppm CO₂ respectively, but no difference at 1200 ppm. Clearly Rubisco concentration and growth are inherently linked tothe atmospheric CO₂ levels. Increases in CO₂ levels have been shown to cause a decrease in leaf Rubisco levels. It has been hypothesized that decreased N investment in Rubisco may lead to an increase in photosynthetic nitrogen use efficiency, and greater biomass production. In line with this, wheat (C_3) plants grown under elevated CO₂ were taller, produced more tillers, had lower leaf area ratio (leaf area per unit shoot mass), and at high N availabilities, had relatively larger root systems than plants grown under control CO₂ levels. CO₂ levels had no effect on maize (C₄) growth in the same experiment, perhaps suggesting that future increases in atmospheric CO₂ levels may have a smaller effect on C_4 plant morphology than for C_3 plants. If these findings can be applied to forage grasses, reduced leaf area and protein content would suggest that increasing CO, levels may lead to significant declines in forage quality in C₃ grasses. "The reality is that as growing season temperatures continue to increase, the yield hit caused by photorespiration will also increase," said Paul South, a USDA-ARS postdoctoral researcher in the, Carl R. Woese Institute for Genomic Biology at the University of Illinois. "If we can translate this discovery to food crops, we can equip farmers with resilient plants capable of producing more food despite increasing temperature stress."

4. Plant Morphology, Photosynthetic N Requirement and N uptake

Plant N concentration decreases allometrically with plant size (Gastal, 2002). The minimum N concentration which allows maximum growth is termed the "critical N concentration", with plants grown at sub-optimal N levels exhibiting proportionately decreased growth rates. Although there are clear differences in critical N concentrations between C_3 and C_4 plants due to the lower N requirements of C_4 plants, the relationship between plant size and N concentration can be found in both groups, with the primary factor appearing to be a decrease in "leafiness"



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through plant development (Gastal 2014). As the plant grows, over time a greater proportion of biomass must be allocated to stems and other support material. Thus, the decrease in N concentration with age may reflect an increase in shaded or senescent leaves over time, or a shift in shoot to root ratio such that C fixation rates are higher than N uptake rates. Given that there is a light gradient down the canopy, with older leaves shaded by younger leaves, plant nitrogen use efficiency can be increased by translocating N in excess of photosynthetic requirements from older or shaded leaves to younger leaves which have an optimal canopy position.

Grasses undergo progressive senescence where the older leaves senesce and are replaced by younger leaves on a continual basis. Photosynthetic rates at ambient CO₂ levels match with leaf Rubisco content, which increases rapidly during leaf emergence, reaches a peak around full expansion, and declines through until senescence. However, even under high exogenous supply, approximately half of the nitrogen for new leaf growth comes from the remobilization of leaf proteins including Rubisco from older leaves. Thus, Rubisco represents a key juncture between carbon and nitrogen metabolic pathways. Rubisco retention in older leaves may increase the photosynthetic capacity of that leaf; however, particularly under N limiting conditions, carbon fixation might have been higher had the N been remobilized to younger leaves. In this case, maximizing leaf photosynthesis may lead to a decrease in carbon fixation at the plant level. Thus, we expect a balance between the maintenance of leaf photosynthesis, and nitrogen remobilization to new tissues. Nitrogen remobilization is known to be important in supporting the development of new leaves in Poa trivialis L. and Panicum maximum (Jacq.), despite removal of the exogenous N supply, new leaves continued to be produced using N remobilized from older organs. Although some loss of N must be expected in senescent biomass, N remobilization is clearly important in determining plant productivity, with a recent paper demonstrating significantly reduced growth in mutant rice plants with reduced capacity for Rubisco degradation, particularly under N limiting conditions. Thus, we can postulate an optimal leaf protein turnover rate which maximises canopy rather than leaf photosynthesis. Given the importance of CO₂ availability to biomass production in Rubisco over-expressing rice, this optimum is likely environmentally modulated, or contingent on other factors, such as plant size. Development of grasses using high catalytic efficiency Rubisco may reduce the need for internal recycling of nitrogen, leading to a more uniform N allocation between leaves.

In a study using short and long-leaved varieties of tall fescue, MacAdam *et al.*(1989) demonstrated that while cell length and the relative leaf elongation rate were genetically controlled, environmental factors such as temperature and light availability, as well as N supply, were important in determining the rate of leaf expansion. Plants receiving high N supply produced a significantly greater number

of mesophyll cells, relative to epidermal cells. This may yield thicker leaves, and given the importance of mesophyll cells for photosynthesis, might partially explain higher rates of photosynthesis in plants grown under high N conditions.

Canopy photosynthesis optimization models predict that under low light availabilities, canopies should have a low leaf area index (LAI; leaf area per unit ground area) which minimizes the self-shading of leaves. Under high light conditions, a greater number of leaves, and higher LAI, are predicted. However, optimized canopy models do not take into account the fact that LAIs which are optimal for canopies may not be evolutionarily stable for the individuals within those canopies, since low LAI plants can be outcompeted by plants with higher LAI, which have higher individual photosynthetic carbon gain. Thus, the optimal LAI for the plant is higher than the optimal value for the canopy. However, there must similarly be a maximum LAI for plants, beyond which the carbon cost of increasing LAI is greater than the photosynthetic benefit. At a given LAI, in the absence of N fertilization, canopy photosynthesis in field grown tall fescue (Festuca arundinacea Schreb.) was 30% reduced relative to well-fertilized plants. Since the measurements were controlled for LAI, these differences were attributed to a direct N effect on photosynthesis (Gastal, 1993). Grass growth is known to be strongly seasonal, and even N-deficient plants grown in spring or summer achieved significantly higher LAI than N-deplete plants grown in autumn (Bellanger, 1994). Presumably, this results from a combination of low temperature, which limits leaf elongation rate (Gastal, 1992), and reduced day-length and light intensity limiting carbon fixation. Taken together, these results suggest that nitrogen availability directly influences shoot growth via LAI, with photosynthetic declines resulting from N-deficiency important only under otherwise optimal conditions. Along with absolute leaf area, specific leaf area (SLA; leaf area per unit dry mass) can also vary and is an important factor in determining plant photosynthetic performance. Anten and Hirose (Anten, 1998) demonstrated that in Xanthium canadense (Mill.), SLA decreased with increasing light intensity, independent of nutrient availability. Similar results were noted in Lolium perenne with comparatively low SLA (160 cm2 g-1) at high light, and higher values (500 cm2 g-1) at low light levels (RA Carnevalli, personal communication). Poorter and Evans showed that plants with high SLA had similar photosynthetic rates to low SLA plants on an area basis, but higher rates on a unit mass basis. At lower light intensities, high SLA plants tended to have higher photosynthetic rates per unit nitrogen, due to a greater leaf area production.

5. Nutrient and Water Availability

Nitrogen deficiency is a major factor limiting the growth and yield of agronomically important plants, with inorganic N fertilizers used as a cost-effective method to

increase crop yields globally. Nutrient capture by roots is a significant cost for plants, both in terms of soil exploration by root production and the C costs of root activities, which includes both nutrient uptake and assimilation. However, plant N content has a primary role in determining whole plant photosynthetic potential, and thus photosynthesis and productivity, and C investment in roots can thus be seen as a method of maximising future photosynthesis. Given the necessary allometric relationships between leaf area and root biomass, correlations between plant N uptake and shoot growth have been confirmed.

Maximum plant growth rates will be achieved when the partitioning of biomass facilitates sufficient nutrient uptake rates to match the rate of carbon supply by photosynthesis. Long-term shifts in canopy photosynthesis (e.g., due to seasonal changes in light availability) have been shown to strongly affect the availability of carbohydrates for the production of leaves, roots, and daughter tillers (Hikosaka, 2005). Thomasand Sadras (2001) have suggested that rather than being carbon limited, most plants have a carbon surplus, and they posit root growth as a mechanism to balance carbon influx with respiratory usage. However, as well as these evolutionary explanations, we should consider more physiological mechanisms for these phenomena. In nitrogen starved wheat plants, approximately equal proportions of the carbon transported to the roots are incorporated into biomass, respired, and translocated back to the shoot as amino acids. As nitrogen is supplied to roots, we might assume that C demand will increase, as a result of the respiratory and C-skeleton costs of amino acid synthesis. Assuming C supply to the roots to be relatively invariant over the short term, this might lead to decreased allocation of carbon for root production. In line with this, Scheible et al. (2001) noted that nitrate accumulation in tobacco shoots was accompanied by a decrease in root sugar content, and that root sugar content correlated with root growth.

Very high levels of N supply tended to retard root growth and branching. Ammonium patches also cause root proliferation, although the roots are shorter and much more highly branched when provided ammonium compared with nitrate. This indicates separate mechanisms for root proliferation by nitrate and ammonium, which may have a functional significance, since nitrate has a diffusibility around 10-times higher than ammonium in soil. Nitrogen availability is known to affect the allocation of biomass to the shoots or roots. However, the N form is rarely considered. Zerihun *et al.* 1998 ,noted that there are significantly different C costs associated with assimilating nitrate, ammonium, or glutamine, and that the physical location and time of assimilation could also have effects. For example, malate derived from photorespiration acts as a source of reducing power for nitrate, where assimilation occurs in the leaves during the light period, without significantly affecting carbon fixation rates. The N assimilation costs have been postulated to have significant effects on biomass partitioning, and indeed fast-growing grass species have been

shown to have lower nutrient assimilation costs than slower growing grass species (Thomas, 2001), although the data did not support this hypothesis in Phaseolus vulgaris. The factors determining the relative cost of N assimilation remain poorly understood, and further research is required to explore the importance of C-costs of nutrient assimilation in determining plant growth rate and productivity. The ETH, Zurich researchers have genetically modified a key variety of rice, making it very efficient at enriching its grains with iron and zinc. As recommended by the Consultative Group on International Agricultural Research (CGIAR), 15 g-1g-1 dry weight (DW) iron and 28 g/g DW zinc in polished grains are required to provide 30% of the dietary estimated average requirement (EAR). In the latest work, the ETH researchers developed rice lines with iron increases equaling more than90% of the recommended iron content and up to 170% of the recommended content for zinc in rice grains (ETH, 2018).

Work in tobacco has suggested a strong positive relationship between shoot to root ratio and leaf nitrate concentration (Scheible 1997), however that relationship broke down under phosphate deficiency or when plants were supplied ammonium, rather than nitrate. Conversely argued that protein concentration, rather than leaf nitrate levels, was primarily important in shoot growth rates (Andrews et al 2015). Furthermore, as well as abiotic factors noted here, root mass allocation can also be affected by biotic factors—hemiparasitic plant infection has been shown to cause an increase in host plant root mass allocation, presumably caused by nutrient abstraction.

It is generally understood that the majority of grass plant roots occur in the surface layers of the soil. A well-developed root system has the capacity to mitigate local variability in resource variability, providing a continuous supply of water and nutrients to leaves, despite the heterogeneous nature of the soil environment. Plants require large amounts of water for photosynthesis, since drought suppresses stomatal aperture leading to depletion of intercellular CO₂ and increased photorespiration.

The plants growing in dry environments may suffer growth retardation as a result of insufficient water availability. Deep roots comprise approximately 30% of the grass root system, and help to maintain access to water even during drought conditions, Hamblin (1987). However in tall fescue, when the surface layer of soil was allowed to dry out but deeper soil had adequate water, carbon allocation to the shoot was decreased by more than 40%, while root production increased in both the upper, drier layer, and the lower layer. Presumably the functional significance of this is to maximize water uptake; however, the physiological mechanism by which it is achieved remains unclear.

6. Competition

The leaf canopy is a dynamic system with a constant turnover of leaves, influenced by environmental conditions. Within a dense stand where plants are competing strongly for light, vertical growth of the plant would be an optimal strategy. However, in more open canopies, vertical growth increases LAI and self-shading. Thus tillering is a preferred strategy, as this increases the number of growing leaves, presumably facilitating a greater increase in whole plant growth rate than could be achieved by an increase in leaf elongation rate alone. Plant C/N ratio is thought to be important in controlling tiller initiation, with nitrogen replete, carbon deficient Carex rostrata (Stokes.) plants producing significantly more daughter stems than carbon replete, nitrogen deficient plants. Similarly, Davies (1971) presented data showing a strong correlation between tiller number and nitrogen application in Lolium perenne, while Gautier et al. (1983) demonstrated that shading caused a decrease in both leaf appearance rates and tiller formation. Within grass canopies, we expect a negative relationship between plant density and mass. At low planting densities, the growth of new tillers fills the space in the canopy, while at high densities plant dry weight decreases, presumably due to competitive effects. Using Lolium perenne plants sown at a range of differing densities. Plant shoot dry mass followed a-3/2 relationship with plant density under high light conditions. However, when the plants were put under heavy shade conditions (83% reduction in light intensity) or when the tiller masses became very large, this relationship shifted from -3/2 to approximately -1. It is thought that at high plant masses, the stand reaches a maximum yield with no further increase in stand mass possible. It is suggested that environmental factors, other than light, influence the maximum potential biomass. Temperature and light quality both seem to be viable candidates with low temperatures linked to decreased leaf appearance rates (Davies, 1983), although other factors such as water availability and nitrogen availability are also important.

7. Carbon Allocation

Photoassimilate is the major substrate for plant growth, thus it is impossible to understand plant performance through understanding of the factors determining assimilate transport and partitioning (Gautier et al, 1999). Phloem is the primary pathway for the transport of carbohydrates from the sites of assimilation (i.e., mature leaves) to the sites of utilization (growth and respiration). However, the direct study of phloem is difficult, requiring the use of radioisotopes with rapid decay rates (e.g., 11C) and expensive equipment, such as positron imaging systems. Carbon flux rates are generally assumed to be a function of the difference in concentration between C-sources such as photosynthesizing leaves, and sinks such as growing leaves, roots or seeds [Durand et al, 1991, Lemair, 1999).

While source strength is fairly easily understood as deriving from photosynthetic C fixation, sink strength is a less well-defined concept. One way to think about it may be as the capacity of phloem companion cells in the proximity of a meristem to unload sugars. An upper limit may be established by the ability of cells to use carbon during division, expansion and differentiation [Lumaire 1999). The 14C labelling studies show the preferential allocation of carbon to young developing roots, with the oldest roots receiving little from the shoots (Mathew, 1997). Given that the older, established roots have a greater biomass and more apical meristems, we might postulate that they have a greater sink capacity than the younger roots. If C allocation is sink-driven, it would be difficult to understand this allocation. It has been hypothesized that the utilization of photo assimilates by young roots close to the shoot renders the supply insufficient for more distant sinks (Matthew, 1997), or it may be that transport resistance between the leaf and the root apical meristem is a key factor determining carbon allocation. Assuming that transport of assimilates through the phloem follows Poiseuille's law, the resistance of C flow down theroot would be proportional to the root's length. Thus, the root tips of longer roots might be expected to receive less photo assimilate than younger, shorter roots, due to their length rather than their age or axial position. Phloem sap viscosity is known to be an important limitation to C export from photosynthesizing Pinus sylvestris L. leaves, Nikinman (2013), suggesting a similar phenomenon might occur in grass roots, although this remains speculative.

Reciprocal translocation of C and N between tillers appears to be common, and is a potential mechanism by which mature tillers can support the growth and development of daughter tillers. Daughter tillers represent a potential C source for root growth and development. However, the degree of resource sharing between tillers appears to vary between species and varieties, and presumably has an environmental component. Clearly, tillering has important implications for plant productivity, although factors influencing the degree of resource sharing between the main stem and daughter tillers remain poorly understood. Carbon fixed by primary tillers was allocated first to the tiller itself, and secondly to main stem axial roots. However, carbon fixed by secondary tillers tended to be retained almost wholly in those tillers, with secondary tillers having little role in supporting main stem roots. Grain yield is a function of biomass accumulation from flowering to physiological maturity (Wr) and the amount of biomass accumulated before flowering and translocated to the grains during grain filling (T). The contribution of T and Wr to rice yield has been widely studied (Weng et al., 1982; Song et al., 1990; Saitoh et al., 1991; Akita et al., 1992). Increased panicle weight and grain filling percentage were positively correlated with T and Wr (Weng et al., 1982), but T was larger in improved varieties and was more effective than Wr in increasing panicle weight. Miah et al. (1996) reported that compared with low-yielding varieties, high-yielding varieties had higher accumulation of assimilates before heading and greater translocation of these



materials during grain filling. Yoshida (1981) stated that high T supports sustained grain growth and stabilizes grain yield under unfavorable weather conditions.

8. Conclusion

While much progress has been made over the last few decades in certain aspects, a great deal of work remains to be done before a comprehensive understanding of the factors controlling plant carbon allocation and plant productivity emerges. Key questions remain regarding the potential diminishment of C₃ grass nutritive value under elevated CO₂ conditions, and whether C₄ plants will gain in importance as a target for forage plant improvement due to their potentially superior stress tolerance under high CO₂ conditions. Recent work in rice has unequivocally demonstrated the importance of Rubisco turnover for plant growth, and that Rubisco turnover rates appear to have a high degree of genetic variability. However, linking leaf behaviour to whole plant performance has yielded little so far, and a more comprehensive approach to the problem seems necessary. The factors controlling tiller formation remain unclear, with most studies in the area relying heavily on correlation and few causative links demonstrated. Both red/far-red ratio and carbon/nitrogen balance appear important, but there is little clarity on the relative importance of these factors. Similarly, the drivers of carbon allocation to different classes of roots remains a key question, which will probably require a combined modelling/experimental approach. Finally, cooperation and competition between tillers in a single plant for N and C remains almost completely unexplored, and represents an important avenue for future research efforts. Although many questions remain, much work has been done since the heyday of forage research in the 1970s. We continue to hope that a more complete understanding of plant carbon metabolism will help us develop higher-yielding, fertilizer-efficient plants. The twentieth century has seen tremendous increase in yield. Evans (1993) indicated that half of the increase can be traced to the improvements through plant breeding and half through agronomy, although each is important on the other. These improvements have brought large social gains, for example in greater food security, lowering malnutrition.

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Single Cell C₄ Photosynthesis: Potential to Introduce in Rice for Increasing Productivity of Rice in Changing Climate

M. J. Baig and P Swain

1. Introduction

Agriculture is the indispensable base of human society, whose nature and productivity are determined by climate and water. Today, the world's population is 7.2 billion, and 5.4 billion live in the developing world where most of the world's existing poverty is concentrated. Over the next 50 years, the world population is expected to increase by about 50% and climate change will probably result in more extreme variations in weather and cause adverse shifts in the world's existing climate patterns. Will climate change help or hinder our efforts to maintain an adequate food supply for the increasing world population of the next century? Could the beneficial effects of increasing atmospheric carbon dioxide (CO₂) on plants (the so-called "CO₂ fertilization effect") counteract some of the negative effects of climate change? Although increase in CO₂ carbon dioxide concentration is likely to be beneficial to several crops, associated increase in temperatures, and increased variability of rainfall would considerably impact food production. Recent IPCC report and a few other global studies indicate a probability of 10 – 40% loss in crop production in India with increases in temperature by 2080 - 2100.

Rice produced from 1 hectare of land in Asia provides food for 27 people, , but by 2050 that land will have to support at least 43 people besides sustaining the adverse effects of changing climate. Feeding the 5.6 billion Asians in the 21st century will

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be possible only with a second Green Revolution to boost yields by 50% using less water and fertilizer. Theoretical models have been used to examine this problem and they suggest that this can be done only by increasing the efficiency with which photosynthesis uses solar energy. Fortunately, evolution has provided an example of a much more efficient photosynthetic system (C_4) than that possessed by rice or wheat (C_3). Maize and Sugarcane are some examples of C_4 plants. Photosynthetic efficiency of rice can be boosted by transforming it from C_3 to C_4 photosynthesis.

Solar energy captured through photosynthesis over the duration of a crop gives it capacity to grow. The upper limit to crop biomass is determined by the laws of thermodynamics and mass conservation. At the limit, the total biomass is simply a function of the total quantity of solar energy captured and the efficiency with which that energy is made available for synthetic processes. Total solar energy absorption is largely a function of canopy architecture and crop duration. The efficiency of energy use is largely determined by photorespiration, dark respiration and losses of biomass that occur owing to senescence. The opportunities for reducing dark respiration are very limited and senescence is essential in terms of recycling essential nutrients from the vegetative portions of the crop to the reproductive ones. There are many evolutionary examples of plants that have eliminated photorespiration by concentrating CO₂ around the photosynthetic enzyme RuBisCO using a fourcarbon acid (C_4) cycle. Plants such as rice that do not have a CO₂ concentrating mechanism to fix CO₂ into three carbon acids (C₃ plants); their photosynthetic rates in hot environments are about half that of C₄ plants. C₄ plants have double the water use efficiency than C₃ plants, and use about 40% less nitrogen to achieve 50% higher yields.

The repeated evolution of C_4 photosynthesis indicates that it should be feasible to create C_4 rice plants by engineering C_4 genes into C_3 rice and replicating strong selection pressure for C_4 traits that we think exist in nature. The development of the C_4 system can be seen as an addition to the C_3 system and it is now clear that the C_3 and C_4 syndromes are not as rigidly separated as was first thought. The enzymes that are prominent in the C_4 pathway also exist in C_3 leaves although with very low activity. More surprisingly, there is a well-developed C_4 pathway in certain locations in C_3 plants: in the green tissue around vascular bundles, and probably in rice spikelet. On the other hand, maize, a thoroughly C_4 plant, has patches of C_3 tissue wherever a mesophyll cell is not adjacent to a bundle sheath cell particularly in leaf sheaths and husk leaves. Some of the wild relatives of rice have C_4 like anatomical features and others may have CO_2 compensation points usually associated with C_3 - C_4 intermediates.

2. Photosynthesis and its Classification

 $6H_2O + 6CO_2 - C_6H_{12}O_6 + 6O_2$

Photosynthesis is the process by which plants use solar energy to produce sugar in which cellular respiration converts into ATP, the "fuel" used by all living organisms. The conversion of unusable solar energy into usable chemical energy is associated with the actions of the green pigment chlorophyll. Primarily the photosynthetic process uses water and release the oxygen so that we can stay alive. The overall reaction of this process is:



2.1. C₃ and C₄ Plants

The difference occurs in the second part of photosynthesis, the Calvin-Benson cycle, which "fixes" CO_2 into carbohydrates. The Calvin-Benson cycle (in "normal", C_3 plants) consists of three processes:

i. The fixation of CO₂ into a 5-carbon "receptor" ribulose 1,5-bisphosphate

(RuBP),, which results in two 3-carbon molecules (a sugar-phosphate called 3-phosphoglycerate, or 3PG), a reaction catalyzed by the protein RuBisCO.

- ii. The reduction of 3PG to form a carbohydrate, glyceraldehyde 3-phosphate (G3P).
- iii. Regeneration of the CO₂ receptor, RuBP.

For every "turn" of this cycle, one molecule of CO_2 is fixed. The problem comes in the first part of the cycle, where RuBisCO is used. RuBisCO can either grab onto CO_2 or O_2 . If it latches onto CO_2 as it should, then the first part of the cycle produces 2 molecules of 3PG, as it should. If it latches onto O_2 instead, then the first part of the cycle produces one 3PG, and one glycolate. Now, C_3 plants have evolved ways to reclaim at least some of the carbons channelled away as glycolate, by feeding glycolate through a peroxisome and a mitochondrion, where it undergoes several transformations and some of it is released back out as CO_2 (photorespiration). However, it reduces the net carbon fixation by about 25%. C_3 plants, accounting for more than 95% of earth's plant species, use RuBisCO to make a three-carbon compound as the first stable product of carbon fixation. C_3 plants flourish in cool, wet, and cloudy climates, where light levels may be low, because the metabolic pathway is more energy efficient, and if water is plentiful, the stomata can stay open and let in more carbon dioxide. However, carbon losses through photorespiration are high.

RuBisCO has about 10x more affinity for CO₂ than it does for O₂, so under normal circumstances this is not a problem. However, on very hot, dry days plants close the stomata in their leaves in order to minimize the loss of water and this interferes with gas exchange as well. As CO₂ is used up by the normal Calvin-Benson cycle, the balance of CO₂:O₂ inside the leaf alters in favor of O₂, and RuBisCO starts to grab it instead. This both slows down photosynthesis and reduces its carbon fixation overall. The C₄ plants have introduced an extra bit into the Calvin-Benson cycle, an extra early reaction that fixes CO₂ into not 3-carbon sugars, but 4-carbon sugars called oxaloacetate (hence the names, by the way, C3 for 3-carbon and C4 for 4-carbon sugars) by plunking CO, onto a different receptor molecule (phosphoenolpyruvate, or PEP) by way of the enzyme PEP carboxylase. PEP carboxylase has two advantages over RuBisCO: it has no affinity for O, at all, and it finds and fixes CO, even at very low CO₂ levels (1-2 μ m). And oxaloacetate has an advantage over 3PG, in low-CO₂ circumstances some of it degrades to form CO₂ again in the mesophyll, the cells which carry CO₂ to RuBisCO. As a result, the C₄ plants can close their stomata to retain moisture under hot, dry conditions, but still keep photosynthesis ticking over at good efficiency. C_{A} plants possess biochemical and anatomical mechanisms to raise the intercellular carbon dioxide concentration at the site of fixation, and this reduces, and sometimes eliminates, carbon losses by photorespiration. C_{A} plants, which inhabit hot, dry evironments, have very high water-use efficiency, so that

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there can be up to twice as much photosynthesis per gram of water as in C_3 plants, but C_4 metabolism is inefficient in shady or cool environments.

2.2. Crassulacean Acid Metabolism (CAM) Plants

CAM plants (from "crassulacean acid metabolism", because this mechanism was first described in members of plant family Crassulaceae) 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 happen in the same cells, but separated in time (temporal separation). 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. Then, 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.(fig.1)



Fig. 1 Typical C_3 (a), C_4 (b) and CAM (c) plants with carbon fixing cycle

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2.3. Single Cell C₄ Photosynthesis

It has been thought that a specialized leaf anatomy, which is composed of two distinctive photosynthetic cell types (Kranz anatomy) is required for C₄ photosynthesis. C₄ photosynthesis is found to function within a single photosynthetic cell in terrestrial plants. Borszczowia aralocaspica (Chenopodiaceae) doesn't have Kranz anatomy, yet has the photosynthetic features of C₄ plants,. This species accomplishes C, 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 C4 terrestrial plants were discovered recently in two species, Bienertia cycloptera and Borszczowia aralocaspica (family Chenopodiaceae); each has novel compartmentation to accomplish C₄ photosynthesis within a single chlorenchyma cell. C₄ photosynthesis in terrestrial plants was thought to require Kranz anatomy because the cell wall between mesophyll and bundle sheath cells restricts leakage of CO₂. Recent work with the Central Asian chenopods Borszczowia aralocaspica and Bienertia cycloptera show that C4 photosynthesis functions efficiently in individual cells containing both the C4 and C3 cycles. These discoveries provide new inspiration for efforts to convert C₃ crops into C₄ plants because the anatomical changes required for C₄ photosynthesis might be less stringent than previously thought. (Sage, 2002) (fig.2)





3. Will Increased Photosynthetic Efficiency Lead to Increased Yield in Rice?

Plants grown in elevated CO_2 normally have increased yield, which indicates that increasing the availability of photosynthate is likely to increase yield, though not as much as might be expected. In redesigning photosynthesis for increased yield, we can focus on the inputs, fundamental mechanisms, or outputs. Given the importance



of the relationship between photosynthesis and plant growth, focus should also be on the outputs of photosynthesis and their immediate use, especially the enzyme sucrose-phosphate synthase (SPS). Over expression of sucrose-phosphate synthase sometimes have resulted higher yields in transformed plants than untransformed plants. To explain this variability in response, two hypotheses were tested: (1) Does expression of the gene in non-photosynthetic tissue affect yield? (2) Is there an optimum level of SPS that should be sought?. It was found that expression in nonphotosynthetic tissue was not important but that there was an optimum level of SPS activity. Excess or inadequate levels of this enzyme results in lowered yield and it may be that most plants have a level appropriate to the preindustrial atmospheric carbon dioxide concentration or a level that results in a more conservative strategy than is required for crop plants. To elucidate the molecular mechanisms and signaling pathways that control C_4 photosynthesis gene expression, C_4 plants, including maize and sorghum, have been employed. Current evidence suggests that C_4 photosynthesis is advantageous when limitations on carbon acquisition are imposed by high temperature, drought and saline conditions. It has been thought that a specialized leaf anatomy, composed of two, distinctive photosynthetic cell types (Kranz anatomy) are required for C_4 photosynthesis. There are evidence that C_4 photosynthesis can function within a single photosynthetic cell in terrestrial plants. Despite lacking Kranz anatomy, Borszczowia aralocaspica and Bienertia cycloptera (Chenopodiaceae) have the photosynthetic features of C_4 plants. These species accomplish C4 photosynthesis through spatial compartmentation of photosynthetic enzymes, and by separation of two types of chloroplasts and other organelles in distinct positions within the cell cytoplasm.

The single cell C_4 photosynthetic system has given us the impetus that it may be experimentally feasible to genetically engineer all C_4 genes in single cell of rice to enhance its photosynthetic activity and productivity. Our concept in transferring the genes from single cell C_4 photosynthesis system envisages to overexpress phosphoenolpyruvate carboxylase (PEPcase) and carbonic anhydrase (CA) in cytosol, and target pyruvate orthophosphate dikinase (PPDK), NADP-Malate dehydrogenase (NADP-MDH) and NADP-Malic enzyme (NADP-ME) to chloroplasts of C_3 plants using appropriate promoters and vectors. This may lead to improved CO_2 concentrating mechanism in a single cell favouring carboxylation over that of oxygenation function of RuBisCO.

3.1. Photosynthetic Performance of Transgenic Rice Plants Over expressing Maize C_4 Photosynthesis Enzymes

 C_4 plants such as maize and sorghum are more productive as compared to C_3 rice and wheat, because C_4 plants are 30-35% more efficient in photosynthesis. Mutsuoka *et al.* (2001) tried to alter the photosynthesis of rice from C_3 to C_4 pathway by



introducing cloned C4 genes from maize which regulates the production of enzymes responsible for C₄ photosynthesis. Molecular cloning of C₄ specific Pepc (encoding phosphoenolpyruvate carboxylase, PEPcase) gene of sorghum and its high level expression in transgenic rice were studied by Zhang et al. (2003). Characterization and functional analysis of phosphoenolpyruvate carboxylase kinase genes in rice was done by Fukayama et al. (2006). Nomura et al. (2005) studied the differential expression pattern of C_{A} bundle sheath expression genes in rice. Fukayama *et al.* (2003) studied the activity regulation and physiological impacts of the maize C_4 specific phosphoenolpyruvate carboxylase over production in transgenic rice plants. Hiroko et al. (2001) studied the high level expression of C₄-Specific NADP-Malic enzyme in leaves and impairment of photoautotrophic growth in a C₃ plant, rice. The chloroplastic NADP-malic enzyme (NADP-ME) is a key enzyme of the C_{4} photosynthesis pathway in NADP-ME type C_4 plants such as maize. To express the chloroplastic NADP-ME in leaves of a C₃ plant, rice, full-length cDNAs encoding the rice C_3 -specific isoform and the maize C_4 -specific isoform of the enzyme were expressed under the control of the rice CAB: promoter. Transformants carrying the rice cDNA showed the NADP-ME activities in the leaves less than several-fold that of non-transformants, while those carrying the maize cDNA showed activities up to 30-fold that of non-transformants or about 60% of the NADP-ME activity of maize leaves. These results indicate that expression of the rice C_2 -specific NADP-ME is suppressed at co- and/or post-transcriptional levels by some regulation mechanisms intrinsic to rice, while that of the foreign C₄-specific isoform can escape from such suppression. Under natural light, accumulation of maize C₄specific NADP-ME led to bleaching of leaf color and growth was hindered in rice plants. Increased levels of NADPH inside the chloroplast due to the action of the maize enzyme resulted in enhanced photoinhibition of photosynthesis causing such deteriorative effects (Hiroko et al., 2001). C₃ plants including many agronomically important crops exhibit a lower photosynthetic efficiency due to inhibition of photosynthesis by O_2 and the associated photorespiration. C_4 plants had evolved the C_{4} pathway to overcome low CO_{2} and photorespiration. Introduction of the maize intact phosphoenolpyruvate carboxylase gene (Ppc) caused 30-100 fold higher PEPC activities than non-transgenic rice. These results demonstrated that intact C_4 -type genes are available for high level expression of C_4 enzymes in rice plants. (Makoto et al., 1998). Pyruvate orthophosphate dikinase (Pdk) is another key enzyme in C₄ photosynthesis. Based on the results with transgenic rice plants, it was demonstrated that the regulatory system controlling the Pdk expression in maize is not unique to C_4 plants as rice (C_3 plant) also possess a similar system. C_4 photosynthesis is advantageous when limitations on carbon acquisition are imposed by high temperature, drought and saline conditions.

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Transgenic rice plants overexpressing maize C₄-specific phosphoenolpyruvate carboxylase (PEPC) revealed a higher photosynthetic rate (up to 30%) and a more reduced O₂ inhibition of photosynthesis than untransformed plants. There is a small increase in the amount of atmospheric CO₂ being directly fixed by PEPC. Similarly, transgenic rice plants overexpressing the maize chloroplastic pyruvate orthophosphate dikinase (PPDK), also have higher photosynthetic rates (up to 35%) than untransformed plants. This increased photosynthetic capacity is at least in part due to an enhanced stomatal conductance and a higher internal CO₂ concentration. By using conventional hybridization, maize PEPC and PPDK genes have been integrated into the same transgenic rice plants (Ku et al., 2000). In the segregating population, the photosynthetic rates of plants with high levels of both maize enzymes are up to 35% higher than those of untransformed plants. Under full-sunlight conditions, the photosynthetic capacity of field-grown PEPC transgenic rice plants is twice as high as that of untransformed plants. PEPC transgenic plants consistently have a higher photosynthetic quantum yield by photosystem II and a higher capacity to dissipate excess energy photochemically and nonphotochemically. Preliminary data from field tests show that the grain yield is about 10-30% higher in PEPC and 30-35% higher in PPDK transgenic rice plants relative to untransformed plants. Taken together, these results suggest that introduction of C4 photosynthesis enzymes into rice has a good potential for enhancing the crop's photosynthetic capacity and yield.

4. Conclusion

Most of our conventional crops, including rice and wheat, assimilate atmospheric CO_2 by the C_3 pathway of photosynthesis and current atmospheric CO_2 levels limit photosynthesis in C_3 plants. Furthermore, photorespiration reduces net carbon gain and productivity of C_3 plants by as much as 40%. This renders C_3 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 "CO₂ pump," the C_4 pathway of photosynthesis, to concentrate atmospheric CO_2 in the leaf and thus overcome photorespiration. Therefore, C_4 plants exhibit many desirable agronomic traits viz., high rate of photosynthesis, fast growth, and high efficiency in water and mineral use.

There are no closely related C_3 and C_4 crops that we can use to transfer the C_4 traits to C_3 crops by a traditional breeding approach. Thus, research works have been envisaged in engineering the C_4 traits in rice to enhance its productivity. The two important components that needs to be considered while engineering C_4 photosynthesis are: the biochemical pathway (enzymes) and the specialized leaf structure. The coordination of two specialized leaf cells in C_4 leaves (Kranz leaf anatomy), namely mesophyll and bundle sheath cells, is important for pathway function. The C_4 pathway enzymes and their corresponding genes involved in C_4



photosynthesis have been well characterized. However, very little is known about the molecular mechanisms underlying the differentiation of Kranz leaf anatomy in C_4 plants.

Two species namely, *Bienertia cycloptera* and *Borszczowia aralocaspica* (family Chenopodiaceae) have been recently discovered as the dramatic variants of C_4 terrestrial plants; each has got novel compartmentation to accomplish C_4 photosynthesis within a single chlorenchyma cell and the essential features of C_4 are accomplished in a single-cell in contrast to Kranz-type C_4 plants. In a recent work, C_4 photosynthesis was found to function efficiently in individual cells containing both the C_4 and C_3 cycles in the Central Asian chenopods Borszczowia aralocaspica and Bienertia cycloptera. These discoveries have provided new stimulus for the ongoing efforts of converting C_3 crops into C_4 plants because the anatomical changes required for C_4 photosynthesis might be less stringent than previously thought.

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Photosynthesis and PSII Photochemistry under Changing Climate

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1. Introduction

The ever-increasing demand for food crops from the growing population across the world necessitates the concomitant increase in the production. Although a multitude of biochemical and physiological processes govern the growth and development of plants, the photosynthetic capability during the grain-filling stage is a key factor which substantially affects the biomass and ultimately the grain yield in crops (He *et al.*, 2014; Ashraf and Harris, 2013).Environmental stresses such as unfavorable temperature, salinity and drought have profound effects on the physiological and biochemical features of the plant affecting various metabolic functions. Changes in the environmental conditions affect the growth and development of the plant by primarily hampering the photosynthetic carbon assimilation patterns (Reddy *et al.*, 2010).Most of the environmental stress signals act by modifying the ultrastructure of the involved organelles of the photosynthetic machinery, varying the enzyme concentration and inducing modifications in the concentration of various metabolites and pigments associated with it (Ashraf and Harris, 2013).

Photosynthetically active organisms undergoing oxygenic type of photosynthesis harvest light energy and transform it into chemical energy in the form of ATP and NADPH through the transport of electrons inside the complex thylakoid membrane system. The energy is then utilized for assimilation of CO_2 in the Calvin Cycle generating organic carbon in the process and releasing molecular oxygen into the atmosphere (Takahashi and Murata, 2008). Oxygenic photosynthesis primarily depends on two reaction centre complexes, Photosystem II (PSII) and Photosystem I (PSI), connected together by the cytochrome b6f complex andseveral othermobile electron carriers (Govindjee and Whitmarsh, 2010). PSII, the cytochrome b6f

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complex and PSI function in succession, to transfer electrons from water to NADP+, all the time being embedded in the thylakoid membrane. The necessary energy required for driving thisprocessforward is provided by photons of light, which is captured by the antenna systems of both PSII as well as PSI.

Conditions of abiotic stress have been shown to limit the rate of photosynthesis substantially (Yang *et al.*, 2011) leading to circumstances where the rate of absorption of light energy exceeds that of its consumption, necessitating the need for dissipating the excess radiant energy by alternative mechanisms. Avoiding damage to the photosynthetic machinery becomes important and plants initially dissipate the surplus light energy as heat (non-photochemical quenching) but once light stress becomes inescapable, plants endure the stress conditions by focusing the damage to the D1 protein of PSII (reversible photoinhibition). Further, when there is an excessive abundance of surplus energy, D1 turnover is prevented and irreversible photoinhibition occurs (Yamamoto 2016; He *et al.*, 2014).

2. The Response of Chloroplast to Environmental Stress Conditions

Chloroplast being the key site of the photosynthetic process is affected to a greater extent by stressful environmental conditions and is also the prime modulator of the stress response. It has been reported that drought stress of mild intensity may cause to inhibit both photosynthesis as well as stomatal conductance (Medrano *et al.*, 2002). However, severe drought conditions causes dehydration of the mesophyll cells resulting in inhibition of photosynthetic process as well as causes reduction in WUE (Damayanthi *et al.*, 2010; Anjum *et al.*, 2011). Salinity induced damage have been reported to occur in thylakoid membranes (Omoto *et al.*, 2010). Accumulation of high concentration of ions such as Na+ and Cl- disrupt and inactivate electron transport and photophosphorylation of the thylakoid membrane (Mittal *et al.*, 2012). Heat stress, on the other hand, have also been found to cause membrane disruption, thereby inhibiting the activities of electron carriers and enzymes associated with the membranes ultimately leading to a decrease in the photosynthetic rate (Rexroth *et al.*, 2011).

For efficient capture of light energy, chloroplasts often move around in the cell, additionally, inside the chloroplasts, the light-harvesting complexes shiftaround in the thylakoid membrane region to receive and trap light energy. Consequently, when excessive light energy is received, both short as well as long term strategy of avoidance or tolerance mechanisms come into play utilizing the advantagesoffered due to fluidity of the membrane structure.

2.1. Mechanisms for Avoidance of Light Stress

On being irradiated by excessive light, disconnection of PSII core and the lightharvesting complexes of PSII (LHCII) occur along with blockage of energy transfer process from LHCII to PSII core (Yamamoto *et al.*, 2008). In addition, reorientation of LHCII and PSII complexes to decrease energy transfer from LHCII to PSII core also takes place alongside in the thylakoid membranes (Yamamoto *et al.*, 2014). This array of molecular rearrangements assist in dissipating the excessive excitation energy captured in LHCII, upon strong illumination, as heat and is referred to as energy- dependent quenching (qE) in non-photochemical quenching of chlorophyll fluorescence (NPQ; Horton *et al.*, 1996; Niyogi, 1999). This phenomenon has been extensively monitored and studied using pulse amplitude modulation (PAM) fluorometers (Walz, Germany) (Schreiber *et al.*, 1986).

The mechanism involved requires the activation of the xanthophylls cycle by the enzyme violaxanthin deep oxidase (VDE) and protonation of the PsbS protein. Violaxanthin, in the presence of excess light, is released from LHCII and undergoes de-epoxidation to form Zeaxanthin (Fig 1), which, even in its excited state cannot pass on its energy to other antennae chlorophyll molecules leading to dissipation as heat. Reversion to xanthophyll formation by de-epoxidation of zeaxanthin occurs in low light (Kirchhoff, 2014). Membrane fluidity plays an important role in the avoidance mechanism. It has been reported that impairment of qE by mutation of genes encoding the proteins VDE (npq1) and PsbS (npq4) in Arabidopsis causes acceleration of photoinhibition under strong light conditions (Niyogi *et al.*, 1998; Li *et al.*, 2002).





2.2. Mechanisms for Tolerance to Light Stress

2.2.1. Reversible Photoinhibition

With an increase in light intensity, PSII is subjected to severe stress situations making avoidance of the light stress a difficult proposition. Under such circumstances a tolerance mechanism comes into operation, which helps in concentrating the photo-

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induced damage to the reaction center-binding D1 protein (D1) and stimulates the protein turnover (Yamamoto *et al.*, 2016; Aro *et al.*, 1993). The affected PSII-LHCII super-complex undergoes disorganization with replacement of the damaged D1 protein by a new copy .Thus, the photoinhibition occurring under such conditions is a reversible one and is referred to as 'reversible photoinhibition'.

Detailed mechanistic studies carried out at molecular level revealed the major photoinhibition mechanisms of PSII: acceptor- side and donor-side photoinhibitions (Yamamoto *et al.*, 2008). In the acceptor-side photoinhibition process, huge amount of light reduces the acceptor side of PSII to a far greater extent leading to the event of two-electron transport besides the linear one-electron flow that usually occurs (Fig 2A). The first is the consecutive one- electron contribution to oxygen by the reduced acceptor molecules of PSII like Pheophytin (Pheo), reduced plastoquinine (QA-) leading to superoxide radical (O₂.-) generation. The second is the reversed electron flow from Pheo- to the oxidized electron donor P680+ which excites the ground state chlorophyll to the semi-stable triplet state. They then react with molecular oxygen to form the singlet oxygen $1O_2$. As the D1 protein is in close proximity with the singlet oxygen production site, the protein becomes susceptible to photo damage (Yamamoto *et al.*, 2016).

In donor-side photoinhibition, cationic radicals are formed by an endogenous process catalyzed by the inefficient transfer of electrons from the Mn-complex to the oxidized primary electron donor P680+. The P680+ and oxidized secondary electron donor Tyr Z get stabilized but the cationic molecules may cause injury to the D1 protein (Fig 2B). Even weak illumination is capable enough to cause donor-side photoinhibition events.

Disruption of the functional features of the oxygen- evolving step under stress may lead to various ROS generation which may react with the proteins localized nearby in the photosynthetic machinery thereby damaging and degrading them (Pospisil, 2009)



Fig. 2 (A) The acceptor-side photoinhibition mechanism of PSII (B) The donor-side photoinhibition mechanism of PSII (Source: Yamamoto *et al.*, 2016)

Removal of the photo damaged D1 from the PSII complex requires the disassembly of PSII-LHCII super complex and has been studied extensively (Nathet al., 2013). In this process, dissociation of the PSII dimers from the surrounding LHCII trimers is followed by phosphorylation and release of the core protein CP43 carried out by STN8 kinases leading to the disorganization of the PSII dimers enclosing the photodamaged D1 (Aro *et al.*, 2005; Yamamoto *et al.*, 2008). After removal of CP43, the damaged and phosphorylated D1 is dislocated and shifted to the stroma-exposed thylakoid region, where D1 is initially dephosphorylated by phosphatase(s) and then cleaved and removed by protease(s) like ATP- dependent zinc-metalloprotease FtsH (filamentation temperature sensitive H) and ATP-independent serine protease Deg (degradation of periplasmic protein). Following the digestion process, a new copy of the D1 protein is synthesized on the thylakoid bound ribosomes; the elongating polypeptide chain is thereafter inserted directly in the PSII core complex. Subsequent to post-translational assembly, PSII super complex is assembled freshly leading to quick restoration of its function.

2.2.2. Irreversible Photoinhibition

In certain situations, strong illumination causes overproduction of ROS leading to oxidative damage of various protein and lipid molecules and causing the formation of irreversible protein aggregates induced by cross linkages between the damaged D1 and the neighboring proteins. Membrane fluidity and free movement of proteins and lipids are greatly hampered disrupting the function and repair mechanisms of PSII leading to "irreversible photoinhibition". This may eventually result in inhibition of the function of chloroplasts ultimately leading to cell death (Fig 3).



Fig. 3 Irreversible photoinhibition of PSII (Source: Yamamoto et al., 2016)

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3. Effect on Activities of Key Photosynthetic Enzymes

A primary effect of stress is the process of stomatal closure which results in lower accumulation of intercellular CO_2 . Such low levels of substrate cause inactivation of various enzymes involved in key physiological processes.

It was found that increased concentration of salts can adversely affect Rubisco activity (Aragao*et al.*, 2005). Additionally the function of Fructose 1, 6bisphosphatase was found to be regulated by salt stress. While, in case of common bean the function of the enzyme was found to be decreased (Seeman and Sharkey, 1992); Ghosh et al (2001) reported the positive association of Fructose 1,6 bisphosphatase with salt tolerance of rice species.

Similar to salt stress, drought conditions have also been found to influence enzyme activity leading to impaired WUE and carbohydrate assimilation pattern. Rubisco in addition to other photosynthetic enzymes such as PEPCase, NADP-malic enzyme, PPDK was found to be the affected under both mild and severe drought (Du *et al.*, 1998). The decline in Rubisco activity though was shown to be due to alteration in availability of the substrate RUBP, rather than Rubisco per se (Gunasekara and Berkowitz, 1993).

At temperatures higher than optimum, Rubisco activase, the regulatory enzyme of Rubisco is found to dissociate leading to reduction in photosynthetic ability (Raines 2011; Sage and Zhu 2011). Though C_4 plants are generally more tolerant of high temperature conditions yet the photosynthesis carried out in these plants may also be disrupted in elevated temperature conditions (Ashraf and Harris, 2013). It was reported that high temperature may induce impairment of PEP carboxylation and PEP regeneration (Raines 2006, Kelly *et al.*, 2006).

Thus it is found that conditions of stress disturb the optimal balance and activity of various enzymes leading to impaired photosynthetic activity.

4. Conclusion

It is evident thatabiotic stressesmay causeconsiderable damage to the photosynthetic machinery of the plants thereby reducing its performance. PhotosystemII (PSII) is one of the most susceptible components of the photosynthetic machinery that faces the consequences of a stressful environment. Generation of radicals can damage the photosynthetic apparatus, resulting in photoinhibition due to an imbalance in the redox signaling pathwaysand the inhibition of PSII repair (Gururani*et al.*, 2015).Anti-oxidants and detoxifying enzymes are effective tools in the chloroplasts to cope with the stresses (Asada, 2006), whereas the protective processes in the thylakoids are largely dependent on the membrane fluidity of the thylakoids.

Designing plants with improved levels of stress adaptability has proved to be a major challenge forresearchers worldwide. An important goal in this regard would be to elucidate the linkages between theidentified kinases and phosphatases and the influence it exerts on the redox state of the photosyntheticETC. Improvement in photosynthetic performance achieved by overexpression of certain enzymes may pave the way for future research. Elucidation of signal transduction pathways induced by various stress factors may be of vital importance in imparting enhanced tolerance mechanisms. Thus, interdisciplinary research may pave a way towards engineering climate resilient rice crop.

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Photosynthesis and Climate Change Understanding their Interaction through Bioinformatics

Parameswaran, C

1. Introduction

Bioinformatics can be defined as "the science that uses computers and statistical techniques to provide new insights from biological data collections; includes the storage, integration, analysis, and interpretation of biological information, such as sequences of nucleic acids or proteins, as well as experimental data from molecules, gene expressions, and other sources" (Paula da et al, 2018). Thus, the major aspect of bioinformatics understands the biological function of the nucleotides/proteins/ genomes. Global warming due to green house gases is expected to increase the temperature by 1.5 degree Celsius in future (http://www.ipcc.ch/report/sr15/). In addition, frequent occurrence of drought, floods, pest and diseases are also a serious concern for food security of the world. Photosynthesis, comprising of photosystems I and II and other carbon fixation reactions are severely affected by high temperature, drought, salinity, floods, and pest and diseases. Especially, PSII re-assembly during the stress greatly limits the photosynthetic ability of plants (Kato et al, 2018).

2. Growing Influence of Bioinformatics in Photosynthesis Research

The applications of bioinformatics tools are nowadays routinely used by the researchers worldwide for understanding the photosynthesis and effect of climate change on the photosynthetic processes. The validation of endosymbiotic theory related to the origin of chloroplast, plant photosynthetic ability is one of the significant contribution of bioinformatics in understanding the photosynthesis in plants (Martin et al, 2012). An analysis using the keywords such as photosynthesis,

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photosynthesis and climate change, and photosynthesis, climate change and bioinformatics in the pubmed Central (NCBI) showed among the 49,227 publications related to photosynthesis, 1379 publications used bioinformatics tools in their analysis (Figure 1). In addition, the last 2-3 years showed atleast 681 publications which used bioinformatics tools to understand the impact of climate change on photosynthesis (Figure 2).



Fig. 1 The number of publications related to the photosynthesis, climate change and bioinformatics in pubmed central database of NCBI



Fig. 2 The number of publications related to the photosynthesis, climate change and bioinformatics in pubmed central database of NCBI from 1990-2018.

3. Number of Photosynthesis Genes in Arabidopsis Genome

The primary step in understanding the photosynthesis through bioinformatics is identification of number of genes in the plant genome contributing to the photosynthetic processes. A survey was performed in the Arabidopsis TAIR database to identify the total number of genes involved in the photosynthesis. The analysis showed at least 710 genes (Figure 3) are present in the Arabidopsis genome related to the photosynthesis. However, the number is only the genes that are directly involved in the photosynthesis, pentose phosphate pathway genes etc. There are additional genes are involved in the transport of the proteins into chloroplast and membrane dynamics etc. In addition, the number of genes is bound to increase in the crop plants due to the duplication events in the evolution of the monocots and dicots from their ancestors.





4. Rice Data Available in the NCBI Database

The rapid evolution of new sequencing chemistry relative to Sanger sequencing such as next generation sequencing technologies provides enormous volume of data for bioinformatics analysis. NCBI, repository of nucleotide, genome, proteome, and transcriptome data for several organisms assist significantly in the bioinformatics analysis. Thus, the amount of nucleotide data available in the NCBI database for rice was estimated. The analysis showed one thousand billion base pair (Table 1) data of information is available specifically for rice in the NCBI database which

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includes sequence information of nucleotides, genomes and transcriptome data. Therefore, exploring the vast amount of data available for rice could well provide novel understanding of the crop.

5. Databases Available for Bioinformatics Analysis

There are several databases available for the bioinformatics analysis (Table 2). The robust database is the NCBI, which stores multiple information of an organism from bacteria, plants to humans. The data includes ESTs, genes, proteins, transcriptome and epigenome data. TAIR, is the database specifically designed for the Arabidopsis which specifically consist of expression of all the genes in Arabidopsis through eFP browser. RAP-DB and RGAP are the two databases for rice geneome annotation. SNP seek database provides the SNP data of 3000 rice genomes and genevestigator provides expression data for multiple crops. USEGALAXY is the open source bioinformatics analysis tool and ENSEMBL and Gramene are databases specifically designed for the comparative genomics purposes.

6. Abiotic Stress and Photosynthesis

There are several photosynthesis processes affected by abiotic stress in plants. The major process affected is the re-assembly of the PSII due to the abiotic stresses. Also, it is shown that the tolerant varieties synthesize higher amount of D1 protein, a component of PSII in plants. A preliminary analysis of the conservation of the D1 protein in the plants showed very high degree of conservation between the plants including the cyanobacteria. The BLAST N analysis showed there were three homologs of D1 protein in rice. The expression analysis of D1 homologs showed specific up regulation in dehydration and phosphorus limitation conditions (Figure 4).

Oryza Cajanus	1	MTAILERRESTSLWGRFCNWITSTENRLYIGWFGVLMIPTLLTATSVFIIAFIAAPPVDI MTAILERRESESLWGRFCNWITSTENRLYIGWFGVLMIPTLLTATSVFIIAFIAAPPVDI
Azadirachta	1	MTAILERRESERLWGRFCNWITSTENRLYIGWFGVLMIPTLLTATSVFIIAFIAAPPVDI
Arabidonaia	1	MINISERRESESSIONER GNWITSTENRETTOWEGVENTFISSTATSVETTRETAREFVDT
Arabidopsis	1	MTATLERREBESINGRECHWITSTENREITIGWEGVEMTETISTATSVETTAETAAFFVDI MTATLERREBESINGRECHWITSTENREITIGWEGVEMTEMTIGTUNG
Cuanchactaria	1	MATTELERRESESLWORFCHWIISIENREIIOWFGVEMIFILEIRISVFILRFIRAFFVEI MATTELERRESESLWORFCHWITSIENREIIOWFGVEMIFILEIRISVFILRFIRAFFVEI
Nestoc	1	MTATIODDECANUECECNUTTERMINDI VICUECUI MIDTI LAATTOIIIREIRAEEVUI
NUSLOC	1	MAINGARESHAVWEQFCMWIISIMARDIIGWFGVLMIFIDLAAIICFIIAFIAAFFVDI
Oryza	61	DGIREPVSGSLLYGNNIISGAIIPTSAAIGLHFYPIWEAASVDEWLYNGGPYELIVLHFL
Cajanus	61	DGIREPVSGSLLYGNNIISGAIIPTSAAIGLHFYPIWEAASVDEWLYNGGPYELIVLHFL
Azadirachta	61	DGIREPVSGSLLYGNNIISGAIIPTSAAIGLHFYPIWEAASVDEWLYNGGPYELIVLHFL
Hibiscus	61	DGIREPVSGSLLYGNNIISGAIIPTSAAIGLHFYPIWEAASVDEWLYNGGPYELIVLHFL
Arabidopsis	61	DGIREPVSGSLLYGNNIISGAIIPTSAAIGLHFYPIWEAASVDEWLYNGGPYELIVLHFL
Brassica	61	DGIREPVSGSLLYGNNIISGAIIPTSAAIGLHFYPIWEAASVDEWLYNGGPYELIVLHFL
Cyanobacteria	61	DGIREPVAGSLMYGNNIISGAVVPSSNAIGLHFYPIWEAASLDEWLYNGGPYQLVILHFL
Nostoc	61	DGIREPVAGSLLYGNNIISGAVVPSSNAIGLHFYPIWEAASLDEWLYNGGPYQLVIFHFL
0rvza	121	LOUNCYMOREWELSERIGMEDWINUSYCNDUANNAUFLIVDIGOCOFODOMDICIOCOF
Cajanue	121	LOVACINGREWELSPRIGHT CAN DET AVAISAT VAAATAVELTTE TOUGSPENDEN DET STOTE
Azadirachta	121	LOVACYMGREWELSERLGMRPWIAVAYSAPVAAATAVELTYPIGOGSESDGMPLGISGTE
Hibiscus	121	LGVACYMGREWELSFRLGMRPWIAVAYSAPVAAATAVFLIYPIGOGSFSDGMPLGISGTF
Arabidonsis	121	LGVACYMGREWELSERLGMRPWTAVAYSAPVAAATAVELTYPTGOGSESDGMPLGTSGTE
Brassica	121	LGVACYMGREWELSFRLGMRPWIAVAYSAPVAAATAVFLIYPIGOGSFSDGMPLGISGTF
Cyanobacteria	121	IGVFCYMGREWELSYRLGMRPWICVAYSAPVAAATAVFLIYPIGQGSFSDGMPLGISGTF
Nostoc	121	${\tt T}{\tt GVFCYLGREWELSYRLGMRPWICLAFSAPVAAATAVFLIYPIGQGSFSDGMPLGISGTF}$
0	191	NEW TVEOXEUNIT MUDEUMT CUXCUECCET PRAMUCET VIPORT TDEMPENERANE CVDEC
Catanue	191	NEMIVEQAENNIIMHEENNIGVAGVEGGSLESAMHGSLVISSLIKETIENESAMEGIKEG
Azadirachta	181	NEMTVEQAEMNTEMMEETHELGVAGVEGGSDE SAMHGSDVESSDERETTEMESANEGEREG NEMTVEGAEUNTI MUDEUMI GVAGVEGGSDE SAMHGSDVESSDERETTEMESANEGEREG
Hibiecue	1.81	NEMIVEQABILITEREPHILOVAGVEGGSLESAMHGSLVTSSLIRETTENESANAGYREG
Arabidonsis	181	NEMTVEOAEHNTI.MHPEHMI.GVAGVEGGSI.ESAMHGSI.VTSSI.TRETTENESANEGYREG
Brassica	181	NFMIVFOAEHNILMHPFHMLGVAGVFGGSLFSAMHGSLVTSSLIRETTENESANEGYRFG
Cvanobacteria	181	NFMLVFOAEHNILMHPFHMLGVAGVFGGALFSAMHGSLVTSSLVRETTENESONYGYKFG
Nostoc	181	NFMIVFQAEHNILMHPFHMLGVAGVFGGSLFSAMHGSLVTSSLVRETTENESQNYGYKFG
	0.41	
Catanua	241	QLELINIVAANGIPGKLIPQIASENNSKSLHFFLAAWFVVGIWFTALGISTMAFNLNGF
Cajanus	241	QEEETINIVAAHGIPGELIPQIASPNNSESLHPPLAAWPVVGIWPTALGISTMAPNLNGP
Azadirachta Wibicouc	241	QEELTINIVAAHGIEGKLIEQIASENNSKSLHEELAAWEVVGIWETALGISTMAENLNGE OFFFTVNTUAAUCVFCDITEOVACENNSDELUEFTAAWEVVGIWETALGISTMAENLNGE
Arabidoneic	241	QEEETINIVAANGIPGKLIPQIASENNSKSLNPELAAWPVVGIWETALGISIMAPNLNGP OPPERVNTVAANGVEGDITEOVACENNSDETHEFTAAWPVVGIWETALGISIMAPNLNGP
Practica	241	QEEETINIVAANGIFGETEQIASENNODOLUEETANDUNGIWETALGISIMAENLINGE
Cuanchastoria	241	QEEETINIVAANGIPGREIPQIRSPNNSRSENPEERAWPVVGIWPIALGISIMAENLNGP
Nostos	241	QEEETINIVAANGIPGALIPQIASPANSASLIPPLAANDUTGINPATOLOGISIPAPALANG OPPERIVNIVAALGVEGDI TEOVAGENNEDET HEFT AANDUTGINEDAT GUGMAENT NGE
NOSCOC	241	VEPEIINIANAUGIEOKTIEČINSENNOKSPLEEPUNMEATOIMEINPOASIMMENTMOE
Oryza	301	NFNQSVVDSQGRVINTWADIINRANLGMEVMHERNAHNFPLDLAALEVPSLNG
Cajanus	301	NFNQSVVDSQGRVINTWADIINRANLGMEVMHERNAHNFPLDLAVIEAPSING
Azadirachta	301	NFNQSVVDSQGRVINTWADIINRANLGMEVMHERNAHNFPLDLAAIEAPSTNG
Hibiscus	301	NFNQSVVDSQGRVINTWADIINRANLGMEVMHERNAHNFPLDLAAIEAPSTNG
Arabidopsis	301	NFNQSVVDSQGRVINTWADIINRANLGMEVMHERNAHNFPLDLAAVEAPSTNG
Brassica	301	NFNQSVVDSQGRVINTWADIINRANLGMEVMHERNAHNFPLDLAAVEAPSING
Cyanobacteria	301	NFNQSVLDSQGRVINTWADVINRANLGMEVMHERNAHNFPLDLAATEAPSING
Nostoc	301	NENOSVIDSOGRVINTWADTINRANLGMEVMHERNAHNEPLDLAAGDVAPVAISAPAING

Fig. 4 Multiple alignment of D1 protein in plants including Cyanobacteria.

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Fig. 5 Expression analysis of a homolog of D1 gene (LOC_Os10g21192)in rice

SI. No	Name of database	Data available	Selected major applications	Website
1	National Centre Biotechnology Information (NCBI)	Repository of nucleotide, genome and transcriptome data etc.	BLAST analysis, basic genome data, SRA files of expression data	https://www.ncbi.nlm.nih.gov/
2	The Arabidopsis Information Resource (TAIR)	Genetics and molecular biology data forArabidopsis thaliana	Loss-of-function analysis, expression matrix of genes	https://www.arabidopsis.org/
3	Rice Genome Annotation Project (RGAP)	Genome sequence of Nipponbare and annotation of the 12 rice chromosomes	Sequence of genes and proteins of rice can be download	http://rice.plantbiology.msu. edu/
4	Rice Annotation Project Database (RAP-DB)	Genome structure and function of rice based on annotation	Gene sequence can be downloaded using the keyword searches	https://rapdb.dna.affrc.go.jp/
5	Genevestigator	Gene expression data	Expression dynamics of genes	https://genevestigator.com/gv/
6	Ensembl Plants	Genome sequences of several plant species	Comparative genomics	http://plants.ensembl.org/ index.html
7	Gramene	Genome sequences of several plant species	Comparative genomics	http://www.gramene.org/
8	SNP-Seek	Sequence of 3000 rice varieties	SNP identification	http://snp-seek.irri.org/
9	Galaxy	Open source web based bioinformatics analysis	Transcriptome analysis	https://usegalaxy.org/
10	Kyoto Encyclopedia of Genes and Genomes (KEGG)	Molecular level information of genes	Pathway analysis	https://www.genome.jp/kegg/

Table 1	Selected	databases	available	for	bioin	formatics	analysis
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7. Conclusion

The data generation in the crops is expanding at the greater speed than the data utilization. The availability of databases assist in utilizing the sequence information's in the form of nucleotides, expression matrix, SNPs, phenotypes of loss-of-function mutants etc. The bioinformatics analysis assists in understanding the evolutionary differences between the crops and also the response of a variety

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to varied stresses. Also, the use of bioinformatics tools for resolving the research problems are increasing day by day. Thus, the present chapter highlighted the usage of bioinformatics tools for identification of number of homologs and also the expression pattern of the genes in rice. The utilization of basic tools such as BLAST, multiple alignments are crop neutral and can be easily used for formulating a workable research hypothesis.

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Chlorophyll Fluorescence: Basic and Application

R. K. Sarkar

1. Introduction

Chlorophyll fluorescence is widely used as a non-destructive diagnostic tool in photosynthesis research. It is being used to evaluate plant protective responses to stress conditions via rapid, non-intrusive measurements. This tool can be used in plant phenotyping and breeding programs to monitor biotic and abiotic stresses including drought, mineral deficiencies, soil salinity, and pathogenic diseases. This chapter covers different types of instruments available for recording and analysing different types of light signals from the plants to various growth conditions and addresses the use of chlorophyll fluorescence in research on plants. Assuming an antiparallel behaviour between photochemical processes (i.e., light reactions) and non-photochemical processes (i.e., Chlorophyll fluorescence and heat dissipation), photosynthetic activities and physiological state of higher plants, algae, photosynthetic bacteria, etc. are being studied from the time dependent changes of the chlorophyll (Chl) a fluorescence. Phenomena of chlorophyll fluorescence are described.

Photosynthesis has two distinct reactions: light and dark reaction. During light reaction ATP and NADPH are produced. These molecules are used up during dark reaction to produce carbohydrate and other metabolites. The light reaction or precisely called as light dependent reaction starts to operate when light fall on chloroplast molecules. There are two distinct light capturing complexes known as Photosystem II (PSII) and Photosystem I (PSI). The primary acceptor of light is P680 in PSII whereas in PSI it is P700 as they absorb light at 680 nm and 700 nm, respectively. Energy is transported from PSII to PSI to complete the dark reaction. When light fall on chloroplast molecules majority of light energy is utilized to ATP and NADPH production (Oxborough and Baker 1997, Stirbet and Govindjee 2011). Certain light is lost as heat whereas a very less fraction of light is re-emitted as fluorescence during return of chloroplast from excited to non-excited states (Fig.

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1). The Chl concentration is higher in the grana and moreover, as they contain mainly PSII, which leads to more fluorescence than PSI because of the longer fluorescence lifetimes (Strasser and Stirbet 1998, Stirbet and Govindjee 2011). So, analysis of chlorophyll fluorescence of PSII than PSI is more easy and pertinent. Chlorophyll a fluorescence of PSII is known as the signature of photosynthesis of any oxygenic photosynthesis and it has got its importance as to measure the vitality of photosynthetic system this is a very simple method. Chlorophyll a fluorescence emission changes very rapidly due to any perturbation of surrounding environment like both biotic and abiotic stresses.



Fig. 1 Fate of solar energy after absorption by chlorophyll molecules.

The purpose of PSII is to donate electron to PSI. PSII derives the electron from water and supply it to PSI to complete the light reaction. A long series of electron acceptors and donors are involved in the process. Chlorophyll a fluorescence gives an idea whether the system is working to its maximum levels or not?. By measuring the electron acceptor and donor efficiency of Photosystem, structural and functional capability of the system is determined. Each photosystem, has two sides one is the electron acceptor side and the another is the electron donor side (Fig. 2). Chlorophyll a fluorescence precisely determines the electron transport between PSII and from PSII to PSI (Strasser and Stirbet 1998). However, through chlorophyll a fluorescence, electron transport within PSI and beyond PSI is difficult to measure. Hence, we shall discuss the electron movement within PSII system and PSII to PSI.





2. Why Chlorophyll a Fluorescence is so Important?

It tells about the vitality of the plants.

Distinction between tolerant and susceptible genotypes can be made under various abiotic stresses such as submergence, drought, salinity, metal toxicity, nutrient deficiency and several other biotic and abiotic stresses.

This is a high throughput phenotyping technique due its fast and evasive nature.

Chlorophyll a fluorescence is measured mainly through two types of measuring systems. High intensity actinic light is applied to the leaf surface to measure the minimal fluorescence (Fo) as well as maximal fluorescence (Fm). Here, the value of Fo is calculated through the software developed for the purpose. In another model pulse modulated light is used. Fo is determined under very low light intensity. Thereafter, high actinic light is used to measure the Fm. Both types of instruments are in use in chlorophyll a fluorescence studies. Using the values of Fo and Fm several parameters are to be obtained that explain the function of the photosystem. Experimental evidences support that even the extracted parameters are good enough to explain the structural and functional integrity of photosystem. To get the maximal photochemical efficiency of the photosystem II, the samples are put in the dark for 10 to 60 mins depending on the plant materials and sampling time. When high intensity actinic light is used, the oxidation-reduction process goes on simultaneously. Theoretically the reaction centre after dark adaptation is fully in open state or in oxidized state so that it accepts the electron very easily. As time passes there would be the mixed state when both oxidized and reduced electron acceptor and donor molecules exist in reaction centre. Ultimately a situation arises when all the reaction centres are in closed state or in in reduced states. Maximum fluorescence arises during this time and we get the maximal fluorescence (P = Fm). This phasic rise of fluorescence is known as OJIP transients (Fig. 3). This test is known as JIP-test. The Kautsky effect (known as fluorescence transient, OJIP

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curve, fluorescence induction or fluorescence decay) is a phenomenon consisting of a typical fluorescence rise with high intensity of light which was first described in 1931 by H. Kautsky and A. Hirsch (Oxborough and Baker 1997).

The JIP-test formulae are used in extracting the data from the fast chlorophyll a fluorescence rise, their explanation and physiological significances are given in different Tables (Panda *et al.* 2006, Sarkar and Panda 2009).



Fig. 3 O-J-I-P transients, increase of fluorescence intensity with time under high actinic light intensity

3. The JIP-test formulae used in extracting the data from the fast Chl fluorescence rise

QA = First stable electron acceptor in PSII

Vj = (F2ms - Fo)/(Fm - Fo), relative variable fluorescence at the J-step

1 - Vj = ETo/TRo, the efficiency with which a trapped exciton (TRo) can move an electron (ETo) further than QA

ETo/ABS = (TRo/ABSETo/TRo), maximum quantum yield for electron transport towards plastoquinone

ABS/RC = (TRo/RC)/(TRo/ABS), effective antenna size of an active reaction centres (RC)

TRo/ABS = 1 - Fo/Fm, maximum quantum yield for primary photochemistry (efficiency of light reactions)

Some Derived Chlorophyll Fluorescence Parameters

Terms	Formulae	Illustration
Fv/Fm	(1-F0/Fm)	maximal quantum yield of primary photochemistry of PSII
Fv/Fo	[(Fm— F0)/F0]	maximum primary yield of photochemistry (represents activity of the water- splitting complex on the donor side of the PSII)
W	[(F2ms— F0)/ (Fm — F0)]	relative variable fluorescence at J phase of the fluorescence induction curve (reflects the inhibition of electron transport further than QA–)
RC/CS0	(Fv/Fm)*(VJ/M0)* Fo	number of reaction centres per excited cross-section
ETO/CSO	Fv/Fm*(1 – Vj)* Fo	electron transport per unit excited cross-section
PIABS	(R C / A B S) * [Φ P o / (1-ΦPo)]*[ΨEo/(1-ΨEo)]	photosynthetic performance index of PSII on the basis of utilization of absorbed energy (Performance index (potential) for energy conservation from exciton to the reduction of intersystem electron acceptors)
DFABS	log (PIABS)	driving force or PSII redox potential on absorption basis
Area		the space above the fluorescence curve between F0 and Fm; (reflects size of QA, QB and PQ pool)

Chlorophyll fluorescence study gives an account of electron transport within PSII system and transport of electron from PSII to PSI. The following specific parameters give an account of that. Besides, there are some parameters which signify the overall vitality of the photosystem (Goltsev *et al.* 2016, Shasmita *et al.* 2018). All are described below:

i. Parameters Related to the PS II Donor Side

VK = (FK-F0)/(Fm-F0), relative variable fluorescence at the K-step (at 300 µs);

 $\Phi P0 = TR0/ABS = Fv/Fm$, maximum quantum yield of primary PSII photochemistry;

 $\Phi P0 / (1 - \Phi P0) = Fv/F0$, efficiency of light reaction or PSII activity;

ii. Parameters Related to the PSII Acceptor Side

VJ = (FJ-F0)/(Fm-F0), relative variable fluorescence at the J-step (at 2 ms);

 Ψ E0 = ET0/TR0 = 1-VJ, efficiency or probability with which a PSII trapped electron is transferred from QA to QB;

 $\Phi E0 = ET0/ABS = \Psi E0 \times \Phi P0$, quantum yield of electron transport flux from QA to QB of PSII;

 $\Psi EO / (1 - \Psi E0) = (1 - VJ) / VJ$, efficiency of dark reaction;

iii. Parameters Related to the PS I Acceptor Side

N K K I

VI = (FI-F0)/(Fm-F0), relative variable fluorescence at the I-step (at 30 ms);

 $\Psi R0 = RE0/TR0 = 1$ -VI, efficiency or probability with which a PSII trapped electron is transferred from QA - to the end electron acceptors of PS I;

 $\Phi R0 = RE0/ABS = \Psi R0 \times \Phi P0$, quantum yield of reduction in the end electron

acceptors of PS I per photon absorbed;

 $\delta R0 = RE0/ET0$, probability that the electron transferred from the intersystem electron carriers to the electron acceptors at the PS I acceptor side;

iv. Vitality Indexes

 $PIABS = (RC/ABS) \times [\Phi P0/(1-\Phi P0)] \times [\Psi E0/(1-\Psi E0)]$, photosynthetic performance index of PS II on absorption basis;

PITOT = PIABS × [$\delta R0 /(1-\delta R0)$], photosynthetic performance index up to the reduction of PS I end acceptor;

DFABS = log (PIABS), driving force of PS II redox potential on absorption basis; and

DFTOT = log (PITOT), driving force of redox potential up to the reduction of PSI end acceptor.

A wide range of photosynthetic parameters can be derived from PAM fluorometry which gives insight into the physiological state of all photosynthetically active organisms (Ehlert and Hincha 2008, Kalaji *et al.* 2014, Pradhan *et al.* 2018). The parameters are described below:

Parameters	Term / Definition
FO	Dark fluorescence yield when supposedly all the reaction centres are open
FM	Maximal fluorescence yield of dark-adapted sample when supposedly all the reaction centres closed
FV = (FM-F0)	Variable fluorescence
FV/FM	Maximal PS II quantum yield
FM'	Maximal fluorescence yield of illuminated sample
F0'=F0/(FV/FM+F0/FM')	Minimal fluorescence yield of illuminated sample
Ft	Current fluorescence yield
F	Fluorescence yield, average value of Ft in any time
Y(II) = (FM' - F)/FM'	Effective PS II quantum yield
qN = (FM-FM')/(FM-FO')	Coefficient of nonphotochemical quenching
<i>qP</i> = (<i>FM'-F</i>)/(<i>FM'-F0'</i>)	Coefficient of photochemical quenching, measurement of overall openness of reaction centres
qL = qP x FO'/F	Coefficient of photochemical quenching, measurement of the fraction of open PS II reaction centres
NPQ = (FM-FM')/FM'	Nonphotochemical quenching

Symbols and Definition of Chlorophyll Fluorescence Parameters use in Imaging-PAM

Y(NPQ) = 1 - Y(II) - 1/	Quantum yield of regulated energy dissipation
(NPQ+1+qL (FNI/F0-1))	
Y(NO) = 1/ (NPQ+1+qL (FM/	Quantum yield of nonregulated energy dissipation
F0-1))	
$ETR = 0.5 \ x \ Y(II) \ x \ PAR \ x \ 0.84$	Apparent rate of photosynthetic electron transport
µequivalents m—2 s—1	

The development of PAM fluorometry is tremendous. Analyses of images obtained from imaging PAM can easily make distinction between the materials based on their performances under adverse conditions. Kinetic study with time under various light intensity make distinctions better. Both actinic lights based fluorometry and modulated light based fluorometry are useful in stress tolerant studies. Using these parameters, distinction between susceptible and tolerant genotypes is too easy. Now strong information is available in respect of submergence, drought, salinity, metal toxicity, nutrient deficiency, diseases and many more other stresses (Panda *et al.* 2008, Panda and Sarkar 2013, Singh and Sarkar 2014, Sarkar and Ray 2016, Shasmita *et al.* 2018). Large numbers of genotypes / lines can be screened with this technique.

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Theme: III

Rice- photosynthesis/ physiology under abiotic stresses



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Photosynthesis and Rice Productivity under Drought

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1. Introduction

Global climate change currently threatens world food security and is one of the most important challenges facing global rice production which depends on the availability of freshwater (Pandey et al., 2007). Scientists have been pushed to develop varieties of rice that are adaptable to the changing climate to sustain sufficient yield under variable climatic conditions and increasing threats of drought, flood, salinity, and high temperature. Quantification of climate uncertainty is an important indicator for crop yield variation in future climate scenarios. Furthermore, concerns now exist about our ability to increase or even sustain rice yield and quality in the face of dynamic abiotic threats that will be particularly challenging in the face of rapid global environmental change. Along with breeding and agronomic managementbased approaches to improving food production, improvements in a crop's ability to maintain yield with a lower water supply and poor quality of water will be critical. Out of 44.0 million ha area under rice in India, drought is one of the major abiotic constraints and the Eastern Indo-Gangetic plains are one of the major droughtprone, rice-producing regions in the world (Pandey et al., 2005). Of the 20.7 million ha of rainfed rice area in India, around 13.6 million ha are prone to drought. 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

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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. Rice is cultivated in a diverse range of climatic conditions and habitats, but the varieties suitable for lowland areas are more productive than the varieties suitable for upland varieties (Parent et al., 2010). Though rice productivity has increased due to selective breeding since 1965 (Peng et al., 2009), attempts have been made to further increase in grain production through rice cultivation in drought-prone areas to meet the future food demand. Most of the rice varieties cultivated in rainfed upland or low land conditions are developed for irrigated conditions and are susceptible to drought resulting in a loss in crop yield (Swamy et al., 2011). Therefore, development of drought-tolerant varieties has immense significance in rice research that requires an understanding of the key process of primary metabolism that plays a central role in plant performance under drought (Chaves et al., 2009; Lawlor and Tezara, 2009).

Several physiological traits have been reported to be linked to drought tolerance; however, it has been very difficult to combine these physiological mechanisms appropriately to obtain the desired yield increase under drought. Photosynthetic ability has been regarded as important indicator of the growth of plants, because of their direct link to net productivity. Drought hampers rice productivity by restricting photosynthesis, altering the ultrastructure of the organelles and concentration of various pigments and metabolites including enzymes involved in photosynthesis as well as stomatal regulation. The decline observed in leaf net carbon uptake as a result of plant water deficits is followed by an alteration in the partitioning of the photoassimilates at the whole plant level, corresponding in general to an increase in the root to shoot ratio. This is the result of the decline in shoot growth and the maintenance of root growth under decreasing water in the soil (Dash et al., 2017). Such a response is mediated by hormonal control, namely by abscisic acid (ABA), ethylene, and their interactions (Wilkinson and Davies, 2010). The changes in the root-shoot ratio as well as the temporary accumulation of reserves in the stem that occur in several species under water deficits (Chaves et al., 2002) are accompanied by alterations in carbon and nitrogen metabolism in the different organs (Pinheiro et al., 2001), whose fine regulation is still largely unknown. In this context, sugars are likely to be key players in the integration, at the whole plant level, of the cellular responses to internal and environmental alterations. They act as substrates and modulators of enzyme activity in carbon-related pathways and via the control of expression of different genes related to carbon, lipid, and nitrogen metabolism (Rolland et al., 2006). The interplay of sugars with other stress elicitors, such as redox and hormone signals, is at the forefront of present research efforts



(Bolouri-Moghaddam *et al.*, 2010). Sulpice *et al.* (2009) recently suggested that starch is a major integrator of plant metabolism and growth in response to changes in development or the environment, reflecting a regulatory network that balances growth with carbon supply.

2. Effect of Drought Stress on Photosynthesis

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Photosynthesis is the chief metabolic phenomenon of plants that facilitate carbon influx into the biological system. Stomata regulates the entry of CO₂ into the leaves and highly sensitive to high transpiration rate that causes continuous fluctuations in the water potential of the guard cells leads to partial closure of stomata and hence limits the rate of photosynthesis. Stomatal closure occurs much before the drought symptoms appear on the plants. Stomatal closure occurs due to decrease in water potential of the guard cells and through ABA-mediated response that produce to water deficit. Under stressful environment, reduction in transpiration due to stomatal closure also reduce photosynthetic rate (Medrano et al. 2002) and increase in canopy temperature that may damage photosynthetic apparatus but stomatal closure affects more transpiration rate than the photosynthetic rate which is evident from increase in water use efficiency under stress (Sikuku et al. 2010). Change in water potential of mesophyll cells also affects the rate of photosynthesis. Under severe stress, a drastic reduction in CO₂ assimilation rate is noticed due to dehydration of mesophyll cells (Damayanthi et al. 2010, Anjum et al. 2011) and reduction in the efficiency in the utilization of CO₂ by the mesophyll cells (Karaba et al. 2007, Dias and Bruggemann 2010). Stomatal conductance plays crucial role in reducing excessive water loss through transpiration and CO₂ assimilation (Parida et al. 2005, Medici et al. 2007). Thus, stomata closure and mesophyll conductance suppression under moderate and severe drought are solely responsible for decrease in photosynthetic rate (Chaves et al. 2009). Abscisic acid (ABA), a stress hormone (Melcher et al.2009), plays a vital role in the plant growth and metabolism under stress conditions and responsible for stomatal closure. However, the regulation of photosynthesis through both stomatal and non-stomatal entities has been reported (Saibo et al. 2009, Rahnama et al. 2010). Drought also damages photosynthetic pigments and thylakoid membrane substantially under severe stress (Anjum et al. 2011, Kannan and Kulandaivelu 2011). A decrease in chlorophyll content is commonly observed under drought stress (Din et al. 2011).



Fig 1 Photosynthesis under drought stress. Possible mechanisms by which photosynthesis is reduced under stress. (Source: Farooq *et al.*, 2009)

2.1. Effects on Photosystems and Photosynthesis

Photosynthesis is suppressed by both biotic or abiotic factors which are observed through alterations in fluorescence emission kinetics (Baker 2008). Commonly used fluorescence induction parameters such as Fo, Fm, Fv and Fp are the indicators of metabolic disorders subjected to various stresses (Baczek-Kwinta et al. 2011). The Fv/Fm ratio is a measure of maximum quantum efficiency and provides overall photosynthetic performance of the plant (Balouchi 2010). Photosynthetic systems are not generally affected under mild or moderate drought stress as evident from the very less or no change in Fv/Fm ratio during stress and recovery, however, photosystem is severely damaged under severe drought (Zhang et al. 2011). Under severe stress, stomata completely close due to loss of turgidity and CO₂ assimilation stop due to unavailability of CO₂. In such situation, high energy electrons released from chlorophyll molecules oxidize photosystem and other protein complexes in the absence of free NADP molecules causing considerable damage to the oxygenevolving centre of PSII (Kawakami et al. 2009) and D1 protein (Zlatev 2009). Photosystems damaged by stress factors result in reduced light-absorbing efficiency and hence a reduced photosynthetic capacity (Zhang et al. 2011, Zlatev 2009, Vaz and Sharma 2011; Gill and Tuteja 2010, Anjum et al. 2011). Adverse effect of drought stress severely alter electron transport rate that results in decrease in the production of ATP and NADPH.

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The effect of stress on photosynthesis could be stomatal, non-stomatal or the combination of both the factors (Saibo et al. 2009). Though there is a contrasting opinion about whether the impairment of photosynthesis primarily through stomatal or non-stomatal limitations (Dias and Brüggemann 2010, Mafakheri et al. 2010), stomatal regulation has been recognised as the early response to drought (Harb et al. 2010). Due to higher transpiration rate, water potential of the guard cells fluctuates continuously that lead to partial closure of stomata to check excessive water loss from the arial plant parts. During severe drought, stomata completely closed for longer periods that leads to inhibition in photosynthesis (Sausen and Rosa 2010, Pan et al. 2011). Stomatal limitation was generally considered to be the major factor of reduced photosynthesis under water deficit conditions (Bousba et al. 2009). Reduced photosynthesis is mainly ascribed to reduced substomatal CO, concentration. However, distinction between stomatal and non-stomatal limitation of photosynthesis can be evaluated under drought condition based on stomatal conductance (Yu et al. 2008). Photosynthetic rate and internal CO, content are strongly correlated with stomatal conductance (Flexas et al., 2002) depict rate of photosynthesis depend more on CO₂ availability than water content of the leaves (Galmes et al. 2011). However, similar stomatal conductance has been recorded with different leaf water levels under water stress condition illustrate lack of correlation between stomtal conductance and leaf water potential (Peri et al. 2009). It may be concluded that water potential has no effect on photosynthesis until a threshold is reached beyond which a consistent suppression in photosynthesis occurs (Lawlor and Tezara 2009). However, drought tolerant genotypes are generally shown higher Rubisco content per unit leaf area (Kumar and Singh 2009) that lead to higher photosynthetic rates (Bayramov et al. 2010).

2.2. Effects on Activities of key Photosynthetic Enzymes

Photosynthesis is severely altered by reduced intercellular CO_2 caused due to stomatal closure under water deficit condition. Reduced intercellular CO_2 deactivates several enzymes such as Rubisco, sucrose phosphate synthase (SPS) and nitrate reductase (Chaves *et al.* 2009, Mumm *et al.* 2011). Water deficit stress alters activities and levels of different enzymes involved in photosynthesis thus affect water use efficiency of the plant that ultimately reduces crop yield (Gill *et al.* 2011). Rubisco content and activity is generally targeted in breeding programme to increase WUE and crop yield (Hirel *et al.* 2007). Decrease in Rubisco content and activity under drought stress has been observed (Medrano *et al.* 1997), but decline in Rubisco activity under drought solely due to unavailability of CO_2 rather than decline in Rubisco content. So, The main reason for inhibition in photosynthesis is due to decline in chloroplastic CO_2 level or unavailability of RuBP. The extent of decline in photosynthesis due to reduced Rubisco activity under drought condition also investigated using antisense Rubisco (Gunasekera and Berkowitz, 1993).



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Result reveals that 68% decrease in Rubisco activity did not hamper PN under water deficit condition suggesting that drought stress may affect any of the steps involved in the regeneration of RuBP rather than Rubisco itself.

3. Improvement in Photosynthetic Capacity under Drought Stress by Engineering Photosynthesis-related Genes or Transcription Factors

The primary determinant of crop yield is attributed to photosynthesis and the yield potential of a crop lies in the efficacy of the plant to capture light and converts it into biomass (Long et al., 2006). Several pieces of evidences are there that suggest increased yield can be obtained through increased assimilation rate through enhanced CO₂ uptake (Weigel and Manderscheid, 2012). This can be achieved by manipulating enzymes involved in Calvin benson cycle and photorespiratory pathway or transferring genes involved in C₄ photosynthetic pathway. Overexpression of Sedoheptulose-1,7,-bisphosphate (SBPase) has resulted in increased carbon assimilation and biomass yield (Simkin et al., 2017a; Ding et al., 2016) suggesting that SBPase is one of the enzymes that regulate the carbon flow in the Calvin-Benson cycle. Over- expression fructose-1,6-bisphosphatases also resulted in 15% increase in photosynthetic rate (Tamoi et al. (2006). These individual gene manipulations have demonstrated that increases in the activity of enzymes of the CB cycle can increase photosynthetic carbon assimilation, enhance growth, and lead to significant increases in vegetative biomass under controlled conditions.

In addition to CO_2 fixed by Rubisco that results in an influx of CO_2 to Calvin Benson cycle another competing reaction carried out by Rubisco that results in 25% loss of carbon as CO_2 instead of being incorporated in to RuBP (Peterhansel *et al.*, 2010) thereby reducing the efficiency of CO_2 assimilation and impacting yield significantly (Walker *et al.*, 2016). For these reasons, photorespiration has been a long-standing target to attempt for improving photosynthesis. As reviewed recently, there has been an array of approaches aimed at engineering photorespiration with the goal of increasing crop productivity (South *et al.*, 2018). Attempts are being made to down regulate or knock down the enzymes of photorespiratory pathway but those results in growth retardation and lower CO_2 assimilation rates under drought stress (Shimada *et al.*, 2008). Therefore, the concept of stimulating Calvin Benson cycle through down regulation of photorespiratory pathway genes is greatly abandoned and most of the resent approaches focus on alternative pathways that can use CO_2 liberated from metabolism of 2-phosphoglycerate in the Calvin Benson cycle (South *et al.*, 2018).

Several attempts have been made to improve photosynthetic capacity by transferring C_4 traits to C_3 plants. However, attempts are made to transfer C_4 traits to C_3 plants through classical hybridization, but most of the hybrids were infertile (Brown and
Bouton 1993). With the advancement of recombinant DNA technology now it is possible to transfer and express C_4 genes in appropriate places within the leaves of C, plants (Kajala et al. 2012). Activity of maize PEPC gene has been increased to 110-fold compared with the non-transgenic plants rice has been reported (Ku et al., 1999). Similarly, a 40-fold over expression of C₄-Pdk gene (Fukayama et al. 2001), and 70-fold over-expression of C₄-specific NADP-ME gene has been reported in transgenic rice compared to non-transgenics (Tsuchida et al. 2001). Transgenic maize line with enhanced expression of C₄-PEPC resulted in maize 30% increase in WUE and a 20% increase in dry biomass under moderate water deficit conditions (Jeanneau et al. 2002). Introgression of HARDY (HRD) gene in rice has resulted in improved CO₂ assimilation with reduced transpiration rate (Karaba et al., 2007). Introgression of constitutively expressing ABP9 transcription factor confers drought tolerance (Zhang et al. 2008). Although over-expression of several C4 genes resulted in enhanced photosynthetic capacity, but results are not so significant when these genes are transferred to phylogenetically distant plant species (Matsuoka et al., 2001). Furthermore enhancing photosynthetic rate CO, concentrating mechanism in C₃ crops by bypassing photo-respiratory pathway require anatomical structures that along with localization of C₄ photosynthetic enzymes in appropriate leaf tissue. Such alterations has not been achieved so far in C_3 crops, so the development of specific structure parallel to C_4 anatomy through gene manipulation is required rather than only expressing C4 genes in C3 plants. This is the major drawback for achieving little success in developing C₃ crops with C4 traits. Engineering of transcription factors involved in photosynthesis is another promising approach for increasing photosynthetic capacity. Attempts are also made to increase photosynthetic capacity through engineering of a number of transcription factors involved in photosynthesis. One of the transcription factor LONG HYPOCOTYL 5 (HY5), involved in the regulation of gene expression of small subunit of Rubisco (RbcS1A) has a significant role in abiotic stress tolerance (Lee et al. 2007). Similarly, over expression of OsMYB4 regulate indirectly the gene expression of photosynthetic genes by stabilizing Rubisco structure under stress (Khafagy et al. 2009).

4. Conclusion

Though improving yield through single or multi gene manipulation has been demonstrated in this review but attaining future food demand in the present climate change scenario through this single approach is questionable. So, other additional approaches such as reducing NPQ and photo respiratory loss is required by introducing new biosynthetic pathway that bypass this process. Moreover, most of the research conducted on increasing yield focus on increasing source capacity whereas, very few attentions has been given to concomitant increase in sink capacity along with source capacity. Increasing yield potential under drought stress also



require improvement in water use efficiency and nitrogen use efficiency. In order to achieve the future food demand to feed the growing population, new technologies like genome editing approaches for endogenous genes modification and synthetic biology to produce designer promoters and proteins are required.

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-NRRI

Photosynthesis and Productivity of Rice under Submerged Condition

R. K. Sarkar

1. Introduction

Rice is the most important cereal crop for the Asian people. Providing enough food for the world's increasing population is possible through increasing the production of rice under limited natural resources, particularly land and water. Rice is grown in areas where two-thirds of the world's poorest and hungriest live. In the mega-cities of Asia, the poorest of the poor may spend up to 50% of their total income on rice. So, anything that lowers the price of rice will directly benefit hundreds of millions of poor consumers and anything that increases rice productivity will benefit millions of rice farmers and their families. Flooding is one of the major constraints to rice production in many rice growing areas of the world. It is the unpredictable nature of flooding which makes it even more difficult to manage. Changes in the global climate are predicted to alter weather patterns resulting in more frequent heavy storms and sea level rise, which will further aggravate this problem. Submergence or excess water stresses perturb rice growth and productivity greatly in areas were flash-flooding or stagnant flooding are very common (Das et al. 2009). As photosynthesis is the key to sustain life on the earth, likely crop survival and productivity depends heavily on the photosynthetic performances under submergence and water stagnation (Panda et al. 2006, Sarkar and Panda 2009, Sarkar and Ray 2016). Throughout the rainfed rice ecosystems, the amount and seasonal distribution of water supply are considered the most important determinant of productivity (Ramakrishnayya et al. 1999).

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Two main situations prevail in rainfed lowland ecosystem; those are complete submergence and partial submergence. Under complete submergence, rice remains under water for the period of 1-2 weeks due to flash-flooding, whereas under partial submergence (at least 1% of plant part is above water surface) certain part of the plant remains under water for the period of 1 month or more.

2. Photosynthesis under Submerged Conditions

Photosynthesis under normal condition takes place freely with available sunlight, easy transport of gases through air; however, if photosynthesis occurs in underwater it encounters the shortage of light and hindrance in gaseous movement (Ramkrishnayya *et al.* 1998, Colmer and Pedersen 2008). Besides, chances are there that total photosynthetic machinery could be damaged and even plants are unable to perform normal photosynthesis (Panda *et al.* 2006, 2008). Photosynthesis heavily depends on environmental conditions. Light intensity, gas concentrations and gaseous movement are the predominant factors that determine photosynthesis (Pedersen *et al.* 2009, 2013). So, without knowing the environmental conditions under excess water stress, photosynthesis can't be defined accurately.

3. Factors Affecting Photosynthesis under Submergence



The variations in floodwater characteristics across locations induce different

Fig. 1 Plant Experiences various types environmental stresses during complete submergence

responses in various cultivars and hence, conclusions drawn about flooding tolerance at one site cannot be extrapolated to other sites. Flooding from rain water usually results in clear water that generally causes less damage than silted or turbid water. Therefore, a comprehensive understanding of relationship between floodwater qualities is useful to know the photosynthetic characteristics under submergence.

4. Environmental Stresses to which Plants are Exposed During Complete Submergence

4.1. Light and Turbidity of Flood Water

Light reaching the leaves of submerged plants is attenuated by water, dissolved organic matter, silt and/or phytoplankton suspended in the water column. In India, during flash floods the floodwater is turbid. Thus, only a meagre amount of solar radiation reaches at canopy level limiting the capacity of plants to photosynthesize (Ramkrishnayya et al. 1998, Das et al. 2009). Underwater light regime is a major controlling factor of CO₂ and O₂ concentration and hence, affects greatly the physiological status of submerged rice plants (Fig. 1, Table. 1). Diurnal variations in dissolved CO₂ commonly occur in floodwaters, with a build-up of CO₂ during darkness and depletion to concentrations well below those in air-saturated water when light is available for photosynthesis (e.g. 0.003 mol m-3 in flooded rice fields). The dissolved oxygen level and pH of the water in a rice field are positively correlated since the O₂ concentration is largely a result of photosynthetic activity that uses up carbon dioxide and hence, reduces the dissolved CO₂ (and thus H+ concentration), effectively raising both pH and the O₂ levels. Thus, increased level of O2 determines the lowering of CO2 level in floodwater. Under turbid conditions plant survival is decreased severely due to shortage of oxygen and greater depletion of carbohydrates. It was observed that a minimum level (88-184 μ mol m-2 s-1) of light was required to check the senescence under submerged conditions (Setter et al. 1995, Das et al. 2009). Low O₂ concentrations (hypoxic) as well as super saturated level did not protect the plants against senescence.

Factor	Values
Dissolved gases in floodwater	
(5 to 50 cm water depth)	
Oxygen (mol m-3, air saturation at 30oC is 0.24 mol m-3)	0.00-0.60
Carbon dioxide (mol m-3, air saturation at 30oC is 0.01 mol m-3)	0.004-2.000
Irradiance (μ mol m-2 s-1)	
Above water surface	400-2050
At 50 cm water depth	0-35% of above water
рН	6.6-9.7

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Table 1 Environmental characteristics of flood water of rice fields in Eastern India

4.2. Temperature

Increase in temperature greatly affects respiration rather than photosynthesis. However, temperature regime has great impact on the maintenance of photosynthetic system under submergence. Pigment content, chloroplast structural and functional ability is more preserved at 25°C compared to 30°C under submergence (Das *et al.* 2009).

4.3. Gas Diffusion

Gases are known to diffuse 10,000 times slower in water than air. The concentration of O_2 in floodwater during flash floods is generally high but floodwater may become anoxic in some environments, especially during the night when the O_2 produced during the daytime is consumed for respiration (Setter *et al.* 1995). The CO_2 concentration in floodwater during turbulent flash floods tends to be in equilibrium with that in the air due to rapid mixing. Oxygen concentration was found to range between 0.0 and 0.6 mol m⁻³ (air equilibrium 0.24 mol m⁻³ at 30°C), CO_2 concentration varied between 0.004 and 2.00 (air equilibrium 0.01 mol m⁻³) at flooded conditions at different parts of eastern India (Table. 1).

4.4. pH

pH of the flood water tells us about the CO_2 levels of it. CO_2 concentration is abundant at floodwater pH of 5.0, whereas at pH 8.0 availability of CO_2 is greatly decreased (Setter *et al.* 1995, Ramakrishnayya *et al.* 1999). Underwater photosynthesis is better at pH 5.0 compared to pH 8.0 (Table 2).

Table 2 Reduced flood water pH improves survival % in rice under submergence due to better underwater photosynthesis at pH 5.0.

pН	CO ₂ concentration	Survival (%)		
	(mol m ⁻³)	IR 42	FR 13A	
5.0	0.96	62±4	100±0	
6.9	0.31	17±4	100±0	
8.0	0.02	0±0	100±0	

5. Photosynthetic Structure and Function under Submergence Condition

5.1. Protection of Chloroplast Structure and Function

Under natural flooding, availability of radiant energy at canopy level is almost zero, plant receives lower radiant energy enhancing senescence, which affects the photosynthetic apparatus. We suggest that maintenance of chloroplast integrity could give a better option in predicting the survival due to submergence stress (Panda *et*

al. 2006, Sarkar and Panda 2009, Sarkar and Ray 2016). Under mild submergence stress the donor side of PS II was more affected than the acceptor side whereas under severe submergence stress both the donor and acceptor sides of PS II were severely affected by the inactivation of oxygen evolving centre (OEC) with impairment of electron transport chain. The maximal chlorophyll a fluorescence yield Fm refers to complete reduction of PS II acceptor QA, the quencher of fluorescence. This faster rise could be ascribed to a slowdown of electron transport beyond QA- and a smaller pool size of electron acceptors between PS II and PS I. Depending on the light reaction of photosynthesis due to submergence stress chloroplast structural and functional aspects are judged to determine the vitality of photosynthetic system. The genotypes are more competent to protect the photosynthetic structure are more able to counter submergence stress.

5.2. Underwater Photosynthesis

Rice leaf is hydrophobic in nature. While contacting with water a thin film of gases is formed between the leaf and water (Raskin and Kende 1983, Mommer and Visser 2005). Until the film exists rice can continue underwater photosynthesis in a better manner due to free movement of gases between the two media (Winkel *et al.* 2014, Herzog *et al.* 2018). Flooding with turbid water restricts the entry of sunlight into the water, if the light intensity is zero it is obvious to draw conclusion that no photosynthesis occurs. However, with time silt is settled down, water becomes clear and certain amount of light reach the canopy level (Das *et al.* 2009). Rice plant starts photosynthesis. The rate of photosynthesis is far below under submergence compared to normal conditions, however, the energy produced due to minimal photosynthesis is helped in survival under water, supply oxygen to the oxygen deprived tissues and restricts the anaerobic fermentation.

Flood water pH ranging from 5-6 maintains greater concentrations of CO_2 for better photosynthesis during submergence. Gas solubility depends highly on temperature. Under chilling or low temperature below 180C tropical rice tended to slow down photosynthesis. Above 30oC solubility of gases decreased substantially. So, under the temperature range of 20-29oC and water pH ranging between 5 to 6 underwater photosynthesis took place in a greater manner but always far below the normal conditions (Das *et al.* 2009). Due to low rate of photosynthesis under submergence requirement of light is also lesser. It was observed that 180-200 μ mol m-2 s-1 light was enough to perform photosynthesis while rice is under submerged conditions. Light intensity above 200 μ mol m-2 s-1 exerts photo-oxidative damage whereas below 180 μ mol m-2 s-1 is not enough to help optimum photosynthesis under submergence.

5.3. Underwater Photosynthesis and Leaf Gas Film

Rice leaf is hydrophobic in nature. Upon submergence the contact between rice

leaf and water does not take place immediately. With time rice leaf losses its hydrophobic character and contact between water and leaf takes place. Loss of hydrophobic nature decreases under water photosynthesis (Winkel *et al.* 2014, Herzog *et al.* 2018, Kurokawa *et al.* 2018). A thin layer of leaf gas film (LGF) acts as a 'gill' of submerged rice plants. It improves gaseous exchange between leaf and surrounding environment, improve underwater photosynthesis and plant survival under submergence. A wax synthesis LGF1 gene is supposed to confer rice leaf hydrophobicity and gas film formation. It was observed that LGF1 gene improved underwater photosynthesis. The plant with LGF1 gene maintained the hydrophobic nature of rice for longer period under submergence (Kurokawa *et al.*, 2018).

5.4. SUB1 and LGF 1

Submergence tolerance gene SUB1 imparts tolerance to submergence (Panda and Sarkar, 2012 and 2013). Rice with SUB1 gene protects the photosynthetic machinery better under submergence compared to the genotype without SUB1. It was observed that FR13A, donor of SUB1, was able to maintain the gas film for longer duration under submergence. Gas film did not persist for longer duration like FR13A in SUB1 recipient line (Winkel *et al.* 2014, Herzog *et al.* 2018). Gas film collapsed early in Swarna-Sub1 compared to FR13A. It shows that SUB1 does not improve the status of leaf gas film under submergence.

5.5. Regeneration Capacity

When rice plants are subjected to flash flooding they need to adapt themselves to two drastic environmental changes: the changes from aerobic to hypoxic conditions during complete submergence and the subsequent changes from hypoxic to aerobic conditions when the flood water recedes (Panda et al. 2008). Therefore, it is pertinent to investigate the physiological processes that are triggered during aerobic \leftrightarrow anaerobic transitions. When the tissues are hypoxic or anoxic the oxygen dependent pathways especially the energy generating systems are suppressed, the functional relationship between root and shoot is disturbed; both carbon assimilation and photosynthetic utilization process are suppressed. A quick regeneration growth following de-submergence is a desirable quality under frequent flooding to sustain productivity. The photosynthetic apparatus suffered greater damage when the plants were exposed to air. The tolerant cultivars, however, adjusted to the new environment quickly, as appeared from the values of Fo (minimal fluorescence), Fm (maximal fluorescence), Fv/Fm (maximal photochemical efficiency) and area above fluorescence curve between Fo and Fm. The susceptible cultivars failed to adjust with the upcoming conditions and the values of these parameters decreased further. Tolerant cultivars maintained a higher level of chlorophyll during submergence and the subsequent period of re-emergence. Besides, tolerant cultivars also maintained greater photosynthesis and rubisco activities (Panda et al. 2008, Panda and Sarkar 2012).



5.6. Photosynthesis under Stagnation of Water

Unlike complete submergence in the case of partial submergence, the problem of photosynthesis is not so serious. Due to hydrophobic nature of the rice a continuum of gas layer is formed between the surface of the leaf and leaf lamina and water (Raskin and Kende 1983). This air layer acts as a 'gill' of the partially submerged leaf. During night plant consumes O_2 and releases CO_2 . CO_2 is rapidly solubilised in water, creates a low pressure zone inside the air layers. First entry of air occurs from atmosphere to air layers. During day time abundant supply of CO_2 takes place through the large liquid-gas interface provided by the air layers. Supplying more CO_2 and deciphering more O_2 into the above-water atmosphere improves the photosynthetic rates in submerged leaves.

6. Conclusion

Rice plant can carry out photosynthetic reaction while under complete submergence provided penetration of light occurs through water up to the leaf of the submerged rice plant. The pH of the flooded water if ranges between 5 and 6, temperature ranges between 20 and 29oC and light intensity ranges between 180 and 200 µmolm-2s-1 photosynthesis becomes optimum under submergence. Gas film produced between water and leaf lamina improves the photosynthesis while rice is under water. Combination of two genes SUB1 and LGF1 can further improve the tolerance level through improvement of underwater photosynthesis.

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Photosynthesis and Productivity of Rice under Salinity Stress

Koushik Chakraborty

1. Introduction

Last few decades have witnessed substantial increase in productivity of food grains, mostly through adoption of intensive agriculture viz. high yielding varieties, precise crop production practices, more efficient crop protection measures etc. Increasing global population with every passing year, needs 87% more production of food crops such as rice, wheat and maize by 2050 over that we are producing today (FAO 2017). Salinity, poses a major threat to agricultural productivity which is causing 4.0-6.3% yield loss annually across the world. The problem is aggravated due to increasing irrigation with sub-optimal quality of irrigation water and more salinization of coastal area due to rise in sea level because of climate change.Salts are component of soil, and originate from mineral weathering, inorganic fertilizers, soil amendments (e.g., gypsum, composts and manures), and irrigation waters. But when salts are present in relatively high amounts, plant growth is adversely affected. Soil salinity is a measure of the total amount of soluble salt in soil (Table 1). As salinity levels increase, plants extract water less easily from soil, aggravating water stress conditions. High soil salinity afflicts about 95 million hectares of land worldwide (Szabolcs 1994) can also cause nutrient imbalances, result in the accumulation of elements toxic to plants, and reduce water infiltration. In India, soil salinity, spread in almost 8.5 m ha area (Table 2), is the factor limiting plant growth and productivity.

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There are two types of salinity, dry land and irrigation salinity. The dry land salinity is classified as either primary or secondary. Primary salting occurs naturally while secondary salting is induced by human activities such as agriculture. Secondary salinity is an insidious

Nature of Soil	USDA Classification			SSSA Classification	
	ECe (dS m-1)	рН	ESP	ECe (dS m-1)	SAR
Normal	< 4.0	< 8.5	< 15	<2	< 13
Saline	> 4.0	< 8.5	< 15	>2	< 13
Sodic	Variable	> 8.5	> 15	Variable	> 13
Saline-Sodic	> 4.0	> 8.5	Variable	<2	> 13

Table 1. Classification of salt affected soil

(Adapted from Handbook of Agriculture, ICAR, 2011)

problem that may be undetected for years until saline discharge is discovered at the soil surface. Salinity through irrigation resembles dry land salinity, except that groundwater accession is induced through irrigation water rather than rainfall alone. Irrigation salinity refers to an accumulation of salt in the plant root zone or on the soil surface, commonly as a result of saline groundwater rising within two meters of the ground surface.

State	Salt affected area (× 1000 Ha)			
	Canal Command	Outside Canal	Coastal	Total
Andhra Pradesh	139	391	283	813
Bihar	224	176	Nil	400
Gujarat	540	327	302	1169
Haryana	455	Nil	Nil	455
Karnataka	51	267	86	404
Kerala	NA	NA	26	26
Madhya Pradesh	220	22	Nil	242
Maharashtra & Goa	446	NA	88	534
Odisha	NA	NA	400	400
Punjab	393	127	Nil	520
Rajasthan	138	984	Nil	1122
Tamil Nadu	257	NA	84	341
Uttar Pradesh	606	689	Nil	1295

Table 2. Extent and distribution of salt affected soil in India

West Bengal	Nil	NA	800	800
Total	3469	2983	2069	8521

(Adapted from CSSRI Reports)

In saline soil, excessive concentrations of Na+ and Cl- impairs absorption of other beneficial ions such as K+, Ca²⁺ that in turn inhibit plant growth and productivity. Maintenance of cellular K+ level and K+/Na+ ratio is still considered the most important factor for salt-tolerance. Plants do vary in their sensitivity to salinity stress. Although being the most sensitive among cereals, having a threshold salinity level of only 3 dS m-1 rice show considerable variability across its different species and also within different genotypes of the same species. 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. 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 to osmotic stress (Roy et al. 2014). Between the two main sub-species of rice, it is observed that Indica is more tolerant than Japonica. Tolerant Indica varieties are good Na+ excluders, absorb high amounts of K+, and maintain a low Na+/K+ ratio in the shoot (Lee et al. 2003).

Based on the ability to tolerate NaCl concentrations, plants can be classified in to two groups; glycophytes or salt sensitive species (which cannot tolerate even mild levels of salinity for longer periods of time) and halophytes or salt tolerant species (which are capable of growing and thriving under high salinity). Primarily, excess salt in the soil decreases the water potential in the rhizosphere region, rendering plants unable to absorb water even in absence of actual limitation of water quantity; a soil condition termed as physiological drought. As a result, many plant processes viz. at the cellular level including cell enlargement, cell division, cell wall properties etc. as well as various leaf parameters such as colour, succulence, necrosis etc., and at whole plant level, shoot/root ratio, growth and yield get affected (Hasegawa et al. 2000). For most of the glycophytes, highly saline growing condition adversely affects germination process, growth, metabolism and overall physiology by causing both ionic and osmotic stresses (Iterbe-Ormaetxeet al. 1998). Salt stress is also responsible for an increased respiration rate, ion toxicity, changes in C and N metabolism (Kim et al. 2004), mineral distribution, membrane instability (Marschner 1986) and permeability (Gupta et al. 2002), decreased biosynthesis of chlorophyll pigments and photosynthetic inefficiency (Munns 2002), all of which collectively leads to impaired economic productivity.

2. Effect of Salt Stress on Basic Plant Responses

High salinity adversely affects germination, growth, physiology and productivity by causing ionic and osmotic stresses and oxidative damage (Iterbe-Ormaetxeet al. 1998). Salt stress is also responsible for an increased respiration rate, ion toxicity (Sudhir and Murthy 2004), changes in C and N metabolism (Kim et al. 2004), mineral distribution, membrane instability (Marschner 1986) and permeability (Gupta et al. 2002), decreased biosynthesis of chlorophyll (Khan 2003) and photosynthetic inefficiency (Munns 2002), all of which ultimately leads to lowered economic productivity (Fig. 1.). Accumulation of sugars and other compatible solutes that can serve as osmoprotectants, stabilizing biomolecules under stress conditions is a common phenomenon. Although use of ions for osmotic adjustment may be energetically more favorable, many plants accumulate organic osmolytes to tolerate osmotic stresses which include proline, betaine, polyols, sugar alcohols, and soluble sugars. Glycine betaine and trehalose act as osmoprotectants by stabilizing quaternary structures of proteins and highly ordered states of membranes. Proline serves as a sink for carbon and nitrogen and a free-radical scavenger, stabilizes sub cellular structures (membranes and proteins) and buffers cellular redox potential.



Fig. 1 Effect of soil salinity in crop plants (Adapted from Chakraborty et al. 2014)

Salinity is a much complex phenomenon rather than a simple escalation in the concentrations of sodium and chloride ions inside plant tissue (Nouri et al. 2017). Apart from Na+ and Cl-, numbers of other cations and anions viz. calcium, carbonates and sulphates may be present in disproportionate amounts and play crucial role and negatively affecting plant growth (Gorham 1992). Simultaneously, certain nutrients (particularly potassium, nitrogen and phosphorus) may be available or present in such low amounts under saline condition that they might hamper proper growth (Chakraborty et al. 2016). The deleterious effects of salinity on plant growth are associated with (1) low osmotic potential of soil solution (water stress), (2) nutritional imbalance, (3) specific ion effect (salt stress), or (4) a combination of these factors (Ashraf and Foolad2007; HanumanthaRao et al. 2016). All of these cause adverse effects on plant growth and development both at physiological and biochemical levels (Munns 2002; Munns and Tester 2008) as well as at the molecular level (Tester and Davenport 2003). Tolerance to saline conditions involves myriad number of physiological processes manifested in numerous levels of organization viz. alterations in gross morphology, tissue partitioning and coordinated control of transport, biological change for maintenance of protein structure and regulated transcriptome level changes (Tester and Davenport 2003).

2.1. Effect of Salt Stress on Growth, Yield and Nutrient Contents

Rice is sensitive to salt-stress compared to other cereals like wheat and barley, yet it is being grown in salt affected soil because of its ability to grow well in standing water, which helps in dilution of salt concentration at the root zone (Munns and Tester 2008). Rice is highly sensitive at early seedling stage and booting/meiosis stage and is quite tolerant at germination, active tillering and maturity stages. Adaptation to salt stress is to adjust with both osmotic and ionic stresses. Initially osmotic stress occupies the main position, whereas with time more salt is absorbed by the plant and ionic stress plays the leading role. Salt stress is cumulative and with time injury symptoms increase. Tolerant rice cultivars like Pokkali either absorbs low levels of Na+ or restricts the movement of Na+ and thereby maintains low Na+:K+ ratio in shoot / leaf and protects the vital tissues (Yeo *et al.* 1990; Singh and Sarkar 2014). The cultivars absorb greater quantities of K+ withstand the salt stress better (Kobayashi *et al.* 2017). Tolerant cultivars further partition the ions in old photosynthetic and non-photosynthetic tissues and try to protect the young and growing parts of the shoots.

Yield of rice was found to be reduced without affecting straw yield under saline condition. The effect of salinity on rice is many fold, leading to inhibition of germination, difficulties in crop area establishment, leaf area development, decrease in dry matter production and delay in seed set (Asch and Wopereis 2001). It has been well documented that the effect of salinity on seedling growth, seedling establishment, grain yield components such as spikelet number, tiller number has

successively lead to a reduction in grain yield (Zeng *et al.* 2003). A wide range of variation has been reported between and within different rice varieties in response to salinity tested for varying floodwater salinity level. Saline floodwater reduced germination rate by around 50% and yield by 80% for the most susceptible cultivar in accordance to the highest salinity level imposed. Further, salinity also resulted in a decrease of the spikelet number per panicle, 1000 grain weight and increased sterility, regardless of the season and development stage (Khatun *et al.* 1995).

2.2. Effect of Salinity on Photosynthesis and Pigment System

Photosynthesis is the most important process affected in plants under saline conditions. Reduced photosynthesis under salinity is attributed to stomata closure leading to a reduction of intercellular CO₂ concentration and to non-stomatal factors also. There is strong evidence of salinity affecting photosynthetic enzymes, chlorophyll and carotenoid (Stepien and Klobus 2006). Decrease in Chlorophyll and carotenoid contents of leaves in response to salt stress is a general phenomenon (Parida and Das 2005). However, an increase in pigment content in Amaranthus sp has also been observed (Wang and Nil 2000). Studies showed wide variations in pigment content depending on salt stress. In quite a few cases, the chlorophyll content was affected by changes in the Chl a/b ratio, which is an indicator of the antenna size of PS I and PS II. The core antenna contains only Chl a, whereas the outer antenna contains both Chl a and Chl b. A higher Chl a/b ratio therefore indicates a smaller antenna size and a lower ratio a larger antenna size. Chlorophyll a, b, total chlorophyll and carotenoid content decrease in response to salinity stress (Ahmad, 2009). Salt stress directly or indirectly affects the photosynthetic functions by changing the structural organization and physio-chemical properties of thylakoid membranes (Alia-Mohanti and Saradhi 1992). Salinity stress reduces quantum yield and Fv/Fm ratio in oat (Zhao et al. 2007).

Salt stress has both osmotic (cell dehydration) and toxic (ion accumulation) effects on whole plant and leaf physiology (Flowers 2004). Salinity reduces the supply of CO_2 to leaves, and further depresses the already low CO_2/O_2 in chloroplasts (Remorini *et al.* 2009). The consequent accumulation of photo-reducing power causes an excess of electrochemical energy in membranes (Zhu 2001). This extra energy is canalized through the Mehler reaction, which generates ROS such as superoxide anion $(O_2.-)$ and hydrogen peroxide (H2O₂) (Herna'ndez *et al.* 2001), ultimately provoking oxidative-stress syndrome (Herna'ndez *et al.* 2000; Baltruschat *et al.* 2008).

2.3. Effect of Salinity on Plant Water Relation

Salinity and water stress have quite similar effects on the growth and cell viability. Salinity causes pronounced decrease in water uptake and plant growth in shoot and root (Misra and Dwivedi 2004). High concentration of salt in the root zone



(rhizosphere) reduces soil water potential and the availability of water, as a result reduction of the water content dehydration at cellular level and osmotic stress are obvious. The increased amount of Na+ and Cl- in the soil-water medium affects the uptake of many indispensable nutrients through competitive interactions and by affecting the ion selectivity of membranes.

The ability of plants to utilize water as well as changes in plant metabolic processes was reduced due to salinity (Munns 2002). Water potential (Ψ) decreased considerably in the 100 mM sodium chloride and sodium sulphate-treated plants due to salinity induced cellular water loss in Phaseolus vugaris (Kaymakanova and Steova 2008). The decrease in the fresh weight of plants after both salinity and drought-induced water stress have been reported for many species as one of the physiological symptoms of stress (Passioura and Munns 2000; Sucre and Sua'rez 2011).

2.4. Effect of Salinity on Electrolyte Leakage and Membrane Stability

Cell-membrane stability, an indicator of the structural integrity, is affected by dehydration and salt stresses (Thomas 1997). Under saline conditions, plasmamembrane leakage (an indicator of cell plasma-membrane integrity) increases and there is a linear relationship between external salinity and membraneleakage rate (Orcutt and Nielsen 2000). Cell-membrane stability using leaf discs in maize, subjected to osmotic stress, showed correlation with the salinity and drought resistance of the whole plant (Simond and Orcutt, 1988). Increase in electrolyte leakage in the leaves of B. juncea with increasing levels of salinity has been reported by Ahmad et al (2009). High salt depositions in the soil generate a low water potential zone making it increasingly difficult for the plant to acquire both water as well as nutrients (Mahajan and Tuteja 2005). Thus, salt stress essentially results in a water deficit condition in the plant and takes the form of a physiological drought. Salt stress causes disruption of ionic equilibrium, influx of Na+, dissipates the membrane potential and facilitates the uptake of Cl- down the chemical gradient, which is evident from the reduction of membrane stability of salt treated plants. High concentration of Na+ causes osmotic imbalance, membrane disorganization, reduction in growth, inhibition of cell division and expansion. High Na+ levels also lead to reduction in photosynthesis and production of reactive oxygen species (Yeo et al. 1998).

2.5. Effect of Salinity on Ionic Homeostasis

Sodium, an integral constituent of our earth crust naturally present in all soil types. At lower concentration Na+ may promote growth in some plants but eventually it becomes toxic to most of the glycophytes when present in high concentration in growing medium (Flowers and Colmer 2008). Both Na+ and K+ share high



similarity in ionic as well as its physicochemical properties, but unlike Na+, K+ are integral part of plant's life and play essential role in growth and development (Schachtman and Liu 1999). Many basic physiological processes, which are essentially dependent on K+ shows impairment due to hindrances in specific transport and interactions K+ 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 in plants (Dietrich *et al.* 2001). Under saline condition, due to prolonged exposure to salt stress plants often inclined to show K+ deficiency symptoms majorly because of reduced uptake by the root tissue and/or lesser K+-retention in different plant parts coupled with a concomitant build-up of tissue Na+ concentration (Munns *et al.* 2002). Thus, under salt stress, it is quite obvious to get plants with stalled growth and metabolism and skewed K+/Na+ in actively growing plant tissues (Shabala and Cuin 2007; Degl'Innocenti *et al.* 2009). Due to such imbalances, several interlinked physiological and biochemical processes are known to be suffered in plants.

Ion ratios in plants are altered mostly by the influx of Na+ through K+ pathways. The similarity of the ionic radii of the hydrated molecule of Na+ and K+ renders the capability of discrimination between them much difficult and hence forms the basis of Na+ toxicity. In vitro protein synthesis necessitates physiological K+ concentrations in the range of 100–150 mM and is inhibited when Na+ concentrations exceeds 100 mM (Cheeseman 2013) as such high concentrations enables Na+ to compete for K+binding sites. Besides, cytosolic enzymes of halophytes are not adapted to high salt levels and exhibit the same level of sensitivity to salt as enzymes from glycophytes do (Flowers et al. 2014). Hence, in response to elevated Na+ concentrations, plants attempt to maintain low cytosolic Na+ concentrations and a high cytosolic K+/ Na+ ratio (Blumwald et al. 2000). The strategies for the maintenance of a high K+/ Na+ ratio in the cytosol include Na+ extrusion and/or the compartmentalization of Na+ in different tissues (mainly in the plant vacuole) for their easy metabolism (Zhu 2003). These are the two critical processes for the detoxification of cytosolic Na+ and osmotic adjustment required to endure salt stress (Blumwald et al. 2000; Chakraborty et al. 2016d).

Regulation of cellular ion homeostasis during salinity stress is critical for plant salt tolerance. The identification of the salt overly sensitive (SOS) pathway in Arabidopsis has revealed components and mechanisms involved in the plant's response to ionic stress. The DNA sequence differences of the component genes and promoters may be responsible for the variation in sensitivity of different species of Brassica to salt stress, as among different species of Brassica; B. juncea was found to be most tolerant towards salinity in terms of morphological and yield attributes followed by B. napus (Islam *et al.* 2001).

3. Plants Adaptive Strategies for Salt-Tolerance

Physiological studies carried out in many crops during salt stress at vegetative stage indicated that stress tolerance trait inversely correlates with shoot Na+ concentration and Na+/K+ ratio (Ashraf 2004; Negrao *et al.* 2011). Different mechanisms associated with salt-tolerance in crop plants include: (1) maintenance of a more negative membrane potential; (2) intrinsically higher H+-ATPase activity; (3) extrusion of Na+ from the cytosol to the external medium; (4) maintenance of mineral nutrient homeostasis, particularly, higher selectivity to K+ and Ca2+ over Na+; (5) scavenging of ROS; (6) accumulation of compatible solutes for osmotic adjustment etc. At the physiological level, salt-tolerance and ion homeostasis is mostly governed by three major strategies in crop plants: I) Na+ exclusion II) K+-retention and III) tissue tolerance/Na+-sequestration (Munns and Tester 2008). Besides ionic homeostasis, there are few other strategies, which plants employ to overcome the ill-effect of salt stress.

3.1. Handling of Oxidative Stress and ROS Scavenging Activities

Salt stress leads to stomatal closure, reducing CO_2 availability in the leaves and carbon fixation, exposing chloroplasts to excessive excitation energy, which in turn increases the generation of reactive oxygen species (ROSs) and induce oxidative stress (Parida and Das, 2005; Parvaiz and Satyawati 2008). These ROS have potential to interact with many cellular components, causing damage to membranes and other cellular structures. However, an elaborate and highly efficient network, composed of antioxidant enzymes and antioxidants, is responsible for maintaining the levels of ROS under tight control (Gao *et al.* 2008).

Salt induced osmotic stress as well as sodium toxicity triggers the formation of reactive oxygen species (ROS) such as superoxide $(O_2^{\bullet-})$, hydrogen peroxide (H_2O_2) , hydroxyl radical (·OH), and singlet oxygen $(1O_2)$, all of these disrupts cellular structures by damaging mitochondria and chloroplasts (Mittler 2002). Plants have developed a series of enzymatic and non-enzymatic detoxification systems to counteract ROS, and protect cells from oxidative damage (Sairam and Tyagi 2004). The Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX) and glutathion reductase (GR) function in detoxification of super oxide and H_2O_2 (Mittler 2002; Kholova etal. 2009).



Fig. 2 Antioxidant enzyme dependent salinity tolerance mechanism in plants (Adapted from Chakraborty *et al.* 2014)

The SOD constitutes the primary step of cellular defence it dismutates O_2 .- to H_2O_2 and O_2 . Further, the accumulation of H_2O_2 is restricted through the action of catalase or by the ascorbate glutathione cycle, where ascorbate peroxidase reduces it to H_2O . Finally, glutathione reductase catalyzes the NADPH dependent reduction of oxidized glutathione (GSSG) to the reduced glutathione (GSH) (Noctor *et al.* 2002) (Fig. 2.). The tolerant and susceptible wheat genotypes show differences in antioxidant activity in response to salinity stress (Sairam *et al.* 2005). Protective roles of the antioxidant enzymes in temperature and salt stress have been reported for a number of plants species (Jaleel *et al.* 2007). The antioxidants ascorbate and glutathione are involved in scavenging H_2O_2 in conjunction with MDAR and GR, which regenerate ascorbate (Horemans *et al.* 2000).

3.2. Osmotic Adjustment and Production of Compatible Solutes

High salinity causes hyperosmotic stress and ion disequilibrium causing secondary effects (Hasegawa *et al.* 2000; Zhu 2001). Plants cope it either by avoiding or tolerating salt stress and are either dormant during the salt episode or there is a cellular adjust to tolerate the saline environment. Tolerance mechanisms can be categorized as those that function to minimize osmotic stress or ion disequilibrium or alleviate the consequent secondary effects caused by these stresses. The chemical potential of the saline solution initially establishes a water potential imbalance between the apoplast and symplast that leads to turgor decrease, which, if severe enough, can cause growth reduction (Bohnert *et al.* 1995). Growth cessation occurs when turgor is reduced below the yield threshold of the cell wall. Cellular dehydration begins when the water potential difference is greater than that can be compensated for by turgor loss (Taiz and Zeiger 1998).

The cellular response to turgor reduction is osmotic adjustment which is achieved in sub-cellular compartments by accumulation of compatible osmolytes and osmoprotectants (Bohnert et al. 1995; Bohnert and Jensen1996). Soluble sugars and starch has been observed to serve as an osmoticum in a number of plants as a response to salinity. An increase in sugar content in shoots has been reported by Amirjani (2011) and also an increased starch content in roots has been observed in the roots of rice which contributes to the osmotic adjustment in the crop exposed to salinity by maximizing sufficient storage reserves to prop-up the primary metabolism (Sakamoto et al. 1998). However, Na+ and Cl- are energetically efficient osmolytes for osmotic adjustment and are compartmentalized into the vacuole to minimize cytotoxicity (Blumwald et al. 2000; Niu et al. 1995). Since plant cell growth occurs primarily because of directional expansion mediated by an increase in vacuolar volume, compartmentalization of Na+ and Cl- facilitates osmotic adjustment that is essential for cellular development. Filek et al. (2012) reported that Na+ may directly or indirectly exert a positive influence on the accumulation of other compounds involved in osmotic adaptation. Such an assumption could explain the greater tolerance to osmotic stress applied in wheat seedlings grown on media containing NaCl.

Exclusion of salts is one of the most prominent mechanisms to encounter salt injury. Besides exclusion and better partitioning of Na+ and Cl⁻ ions, another mechanism which imparts tolerance to salt-stress is tissue tolerance. However, salt tolerant rice cultivars which possess ions exclusion mechanism often do not show tissue tolerance ability (Munns *et al.*, 2016). Osmotic adjustment under salt stress with greater accumulation of Na+ saves energy. So tissue tolerance has another advantage. Recent findings show that variability of tissue tolerance exists among the medium and highly salt-tolerant cultivars in respect of tissue tolerance (unpublished data). Combining different salt-tolerance mechanisms into a single cultivar is feasible.

Salt tolerance mechanism requires osmolytes/compatible solutes accumulation in the cytosol and organelles, where these function as osmotic adjustment and osmoprotection (Rhodes and Hanson 1993). Some compatible osmolytes are essential ions, such as K+, but the majority are organic solutes. Compatible solute accumulation as a response to osmotic stress is a ubiquitous process in organisms as diverse as bacteria to plants and animals. However, the solutes that accumulate vary with the organism and even between plant and genotypes. A major category of organic osmotic solutes consists of simple sugars (mainly fructose, glucose and sucrose), sugar alcohols (glycerol and methylated inositols) and complex sugars (trehalose, raffinose and fructans) (Bohnert and Jensen 1996). Others are quaternary amino acid derivatives (proline, glycine betaine, β -alanine betaine, proline betaine), tertiary amines (1,4,5,6-tetrahydro-2-mehyl-4-carboxyl pyrimidine), and sulfonium compounds (choline sulphate, dimethyl sulfoniumpropironate) (Nuccio *et al.* 1999). Many organic osmolytes presumed to be

osmoprotectants, as their level of accumulation is insufficient to facilitate osmotic adjustment. Glycine betaine preserves thylakoid and plasma membrane integrity after exposure to saline solutions or to freezing or high temperatures (Rhodes and Hanson 1993). Many of the osmoprotectants enhance stress tolerance of plants when expressed as transgene products (Bohnert and Jensen 1996; Zhu 2001). An adaptive biochemical function of osmo-protectants is the scavenging of ROS that are by products of hyperosmotic and ionic stresses, causing membrane dysfunction and cell death (Bohnert and Jensen 1996).

In plant kingdom the organisms ranging from bacteria to higher plants show a strong correlation between increased cellular proline levels and the capacity to survive both water deficit and salinity. The organic nitrogen may serve as the reserve that can be utilized during recovery from salinity. Though proline is synthesized either from glutamate or from ornithine, glutamate is the primary precursor in osmotically stressed cells. The biosynthetic pathway of proline accumulation consists of two important enzymes, viz. pyrroline carboxylic acid synthetase and pyrroline carboxylic acid reductase and transcripts corresponding to both cDNAs accumulate in response to NaCl treatment. Both these regulatory steps are keys in developing strategies for over producing proline in selected plant species.

3.3. Optimization of Ionic Balance and Nutrient Homeostasis

The fundamental basis of the adaptation of plants to salinity stress is the control of transport of ions across the membranes (Hasegawa et al. 2000). In glycophytes, salt exclusion is the predominant strategy of adaptation to saline environment, which depends mainly on the ability of roots to limit transport of sodium (Na+) from root to the leaves and shoot (Yahya 1998). In sorghum (Sorghum bicolor), wheat (Triticum aestivum), barley (Hordeumvulgave L.) and corn (Zea mays) which are glycophytic in nature and where (Na+ exclusion mechanism is present for salt tolerance (Alberico and Cramer 1993). Also, within the plant, Na+ and Cl- are restricted from reaching sensitive organs (Watson et al. 2001). Internal exclusion mechanisms can involve processes such as sequestering salt ions in specialized tissues by removing them from the transport stream (Blom-Zandstra *et al.* 1998; Jeschke 1981) and/or by effectively compartmentalizing Na+ within vacuoles in the stem and leaf (Leigh and Storey 1993). In other word, if Na+ ions are not strongly discriminated against at the root membrane, salinity of the xylem stream will increase under saline conditions. Some of the ions in the transpiration stream could be selectively accumulated by parenchyma cells in the xylem and then retranslocated back to the root via phloem (Orcutt and Nielsen 2000).

Research on salt-stress is widespread covering identification of new genetic resources and their basis of tolerance, unearthing the *QTLs*/genes such as chaperones (Xu and Mackill 1996), glyoxalases (Singla-Pareek*et al.* 2003), SOS1 (Wu *et al.* 2003), NHX

(Fukuda *et al.* 2004), HKT (Ren *et al.* 2005), Hardy (Karaba*et al.* 2007), *QTLSaltol* (Thompson *et al.* 2010), PDH45 (Amin *et al.* 2012), NAC (Parvin*et al.* 2015), and various other genes (Zhang *et al.* 2016; Razzaque*et al.* 2017). The *QTLs* identified till date for seedling stage salinity tolerance among which the most important and effective *QTL* is the *Saltol* (Gimhani*et al.* 2016). *Saltol* mainly controls shoot Na+/ K+ homeostasis. It explained at least 43% of phenotypic variations in shoot Na+ / K+ ratio (Thompson *et al.* 2010). The *QTL* was identified from the cross between FL478 (IR66946-3R-178-1-1, an offspring of salt-tolerant Pokkali) and IR29. The *QTL* was mapped to the position between 10.7 and 12.2 Mb region on the short arm of the chromosome 1. Several high yielding cultivars have been developed by incorporation of the *QTL* '*Saltol*' through marker added selection / back crossing (Gregorio *et al.* 2013; Singh *et al.* 2016).

Perception of salt stress is followed by subtle changes in Ca²⁺ concentration in cytosol of root cells that triggers the SOS pathway (Guo et al. 2004; Chinnusamy et al. 2005). The SOS3 is a myristoylated Ca^{2+} binding protein that recruits SOS2 serine threonine protein kinase to the plasma membrane after binding of Ca²⁺ (Ishitani et al. 2000;Halfter et al. 2000). An alternative regulator of SOS2 activity, SOS3 like Calcium Binding Protein 8 (SCaBP8, a.k.a. Calcineurin B-like CBL10) has been shown to function primarily in the shoots of Arabidopsis while SOS3 expresses predominantly in roots (Quan et al. 2007). SOS2 mediated phosphorylation of SCaBP8 or SOS3-like proteins increase their stability (Lin et al. 2009). The SOS3-SOS2 or SCaBP8-SOS2 complex then recruits SOS2 to plasma membrane to activate downstream SOS1, which functions to extrude excess Na+ from the cytosol (Shi et al. 2000; Qiu et al. 2002; Quintero et al. 2002; Quan et al. 2007; Quintero et al. 2011). SOS4 and SOS5 also play important roles in salt stress tolerance. While SOS4 encodes a pyridoxal kinase that is involved in regulation of Na+ and K+ homeostasis (Shi et al. 2002), SOS5 aids in maintenance of normal cell expansion during stress (Shi et al. 2003). The SOS-mediated salt stress signalling is represented in Fig. 3. The SOS1, SOS2 and SOS3 mutants show similar phenotype and their genetic analysis has helped improve our understanding of the mechanism of salt-stress tolerance in plants (Zhu et al. 1998).



Fig. 3 A coordinated network of Na+ and K+ transport in different plant parts under salinity stress (Adapted from Chakraborty *et al.* 2018)

Other Na+ transporters functioning in salinity tolerance include those involved in intracellular compartmentalization of Na+ into vacuoles, older leaves or leaf sheath, extrusion outside the cell and recirculation of Na+ out of the shoots to be stored elsewhere, for example, in roots or stem cell vacuoles. Vacuolar Na+ sequestration is one of the most energetically efficient mechanisms by which plants achieve turgor maintenance and cell expansion in saline conditions. The NHX type intracellular Na+/H+ exchangers that mediate this process are driven by the differential proton (H+) gradient generated by vacuolar H+ translocating enzymes such as H+ ATPase and H+ PPase. Plant NHX family can be divided into two groups, class I and class II, based on protein sequence and subcellular localization (Rodriguez-Rosales et al. 2009; Pardo et al. 2006). The class-I NHX proteins are located on the tonoplast, where they function as (Na+, K+)/H+ antiporters (Venema *et al.* 2002), while the class-II, NHX proteins are located in endosomal vesicles of plants (Bassil et al. 2011). These proteins maintain K+ homeostasis and function in aiding normal plant growth and development as well as tolerance to salt stress (Pardo et al. 2006). The AtNHX1 gene, the first plant member of the NHX subfamily of intracellular Na+/H+ antiporters from Arabidopsis thaliana was identified based on its homology to animal plasma membrane Na+/H+ antiporters of the NHE family and the yeast ScNHX1 gene (Gaxiola et al. 1999). Overexpression of AtNHX1 in other plant systems led to improved salt stress tolerance (Zhang and Blumwald 2001; Zhang et al. 2001). A different model for the role of NHX transporters has been proposed by Jiang et al.

mutant strain by increasing its K+ content when exposed to salt stress (Xu et al. 2013).

4. Conclusion

Salinity stress is second most important abiotic stress for cultivated crop plants. Salinity affected area is gradually increasing under the scenario of global climate change. The importance of properly understanding and combating this threat to world agriculture is therefore, more apprehended by the researchers. Soil salinity adversely affects plant growth and development accompanied by an increase in uptake of Na⁺ and Cl⁻ ions and a decrease in uptake of K⁺, Ca²⁺, Mg²⁺ resulting in ionic imbalance, sodium ion injury and disturbed metabolic processes, changed concentration of biomolecules, photosynthetic activity and poor productivity. Other most detrimental effect faced by the plants is sudden outburst of reactive oxygen species produced due to salinity stress, which disrupts the cellular structure and damages subcellular organelles, leading to cell death. At molecular level, efficient operation of different signal proteins and various symporters and antiporters lying either in the plasmamembrane or tonoplast play important role in salinity tolerance. Activity of different Na+/H+ antiporters viz. SOS1 and NHX1 depends up on the activation of other signal proteins like SOS2, SOS3 and other calcium binding proteins.

The genotypes having superior antioxidant defence capacity in terms of either accumulation of antioxidants like ascorbic acid, glutathione, malonaldehyde etc. or higher activity of the enzymes are more capable of withstanding salinity stress. Salinity stress cause osmotic and oxidative stress, hence genetic modifications in these areas could yield beneficial result in bringing salinity tolerance in crop plants. Incorporation of genes facilitating biosynthesis of compatible solutes whose accumulation will help in osmotic adjustment in the plant cell thereby maintaining better water balance inside the plant tissues when it is facing osmotic pressure from outside. Though, there is ample opportunity for research in this area, it needs multidisciplinary approaches to address all the component of the problem of salinity.

Adaptation to salinity stress involves osmotic homeostasis, ionic homeostasis, ROS detoxification as well as tissue adaptation mechanisms. These adaptation strategies are governed by a network of several interacting pathways which are controlled by both genetic and epigenetic regulations. In this context, many cultivated accessions were identified with tolerance to salinity stress. *QTLs* for salt tolerance have been identified from those cultivated accessions. A few of these have been identified, mapped, cloned and introgressed into elite varieties using molecular

breeding approaches. Satisfactory progress in transferring tolerance to high yielding cultivars for better survivability is made. But yield penalty under salinity stress can't be reduced significantly. In this context, some of the wild relatives with better tolerance are being utilized in the breeding. The major setback faced by the breeders in this approach is that most of wild tolerant genotypes are often cross-incompatible with the cultivated species. There is a need to go for mining of the differentially expressed genes and subsequent transfer of those to cultivable species.

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Rice Photosynthesis and Productivity under High Temperature Stress

Prashantkumar S Hanjagi and Sushma M Awaji

1. Introduction

Intensiveresearch on climate change in recent times shows that rising temperatures may intensify storms, flooding and other severe weather events worldwide, and eventually affect food production. Without (successful) adaptation, local/ global temperature increases in excess of about 1°C above pre-industrial period is projected to have severe negative effects on yields for the major crops (wheat, rice and maize) in both tropical and temperate regions (IPCC 5th assessment report, 2014). There are research findings highlighting the benefits of increasing atmospheric concentrations of carbon dioxide (CO₂) on crop plants, but simultaneous increase in temperature will probably affect these likely benefits. Climate change is predicted to affect agricultural production the most, primarily at low latitudes populated by developing countries, with adverse effects of increasing carbon dioxide and high temperature, challenging researchers toward devising adaptation strategies (Rosenzweig *et al.*, 2014). These constraints to global food supply and a balanced environment encourage research and development of climate smart crops, resilient to climate change (Wheeler and Von Braun, 2013).

Rice (*Oryza sativa* L.) is an important global food crop and provides food security for many countries. It serves as a primary source of food accounting for about 35 to 75% of the calorie intake of more than 3 billion people. With the projected increase in world's population toward 10 billion by 2050, the demand for rice will grow even faster than for other crops. Rice grows optimally at a temperature ranging from 22°C to 30°C, and the current rise in temperature averages would certainly affect global rice production (Korres *et al.*, 2017). The critical temperature for rice production varies depending on the genotypes and physiological status of the plant but extreme temperature is detrimental to its growth and development (Krishnan *et al.*, 2011)

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(Figure 1). The predicted increase in global temperature could be catastrophic to rice yield and quality when temperatures beyond known critical threshold (33°C) coincide with sensitive growth and developmental stages. High temperature stress causes cell damage by inducing Reactive Oxygen Species (ROS). Temperatures above the optimum for growth (heat stress) may injure and/or irreversibly damage photosynthetic machinery, pigments, and metabolites including photosynthetic enzymes, as well as the stomata (Figure. 2). The toxic effects of ROS under normal physiological conditions, are minimized by enzymic and non-enzymic antioxidants. But, under stress conditions oxidant levels can overcome the antioxidant levels. As a result, inhibition of photosynthesis, metabolic dysfunction and damage of cellular structures contribute to growth perturbance, reduced fertility and premature senescence (Krasensky and Jonak, 2012).



Fig 1: Response of the rice plant to varying temperature at different growth stages (adopted from Yoshida, 1978)

2. Effect of Heat Stress on Photosynthesis

Photosynthetic processes of rice are negatively affected by high temperatures, but to a lesser extent than reproductive development. Heat stress on rice speeds up leaf senescence and damages photosynthetic machinery and pigments. Across the thylakoid membrane, reactive oxygen species (ROS) are produced due to heat stress, leading to oxidative damage. Photosynthesis is limited by severe heat stress which results in reduced activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), phosphoenolpyruvate carboxylase (PEPCase), nicotinamide adenine dinucleotide phosphate malic enzyme (NADP-ME) and pyruvate phosphate dikinase (PPDK). Heat-induced reduction in the activity of Rubisco activase affects Rubisco activity. Vu et al. (2007) found an increase in leaf CO₂ exchange rate (CER) of rice by CO₂ enrichment, but that was decreased by high temperature and drought under elevated [CO₂]. High temperature, elevated [CO₂], and drought have been found to reduce the initial (non-activated) and total (HCO₂-/Mg²⁺ activated) activities as well as the activation state of midday-sampled leaf rubisco. With increases in ambient temperature, stomatal conductance and CO₂ solubility in water decrease with increase in ambient temperature, which not only reduces carboxylation directly but also directs more electrons to form ROS and promotes photorespiration. Moreover, noncyclic electron transport is downregulated to match the reduced requirements of NADPH production, thus reducing ATP synthesis (Figure 1).



Fig. 2 Influence of heat stress on photosynthesis (modified from: Rehman Abdul, 2016).

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2.1. Effect on Leaf Senescence and Photosynthetic Pigments

It has been observed that high temperature accelerates senescence related metabolic changes in plants (Paulsen 1994; Nawaz *et al.* 2013). Heat stress accelerates the chlorophyll degradation and/or reduces rate of biosynthesis of chlorophyll thereby substantially reducing the chlorophyll content (Fig 1). Under heat stress, chlorophyll biosynthesis is reduced which is caused by reduced activities of the enzymes involved in its biosynthesis (Reda and Mandoura 2011), such as 5-aminolevulinate dehydratase (ALAD), the first enzyme in the pyrrole biosynthetic pathway (Tewari and Tripathy 1999; Mohanty *et al.* 2006); protochlorophyllide (Pchlide), oxidoreductase and porphobilinogen deaminase (Tewari and Tripathy 1998).

2.2. Effect on Photosynthetic Machinery

The photosynthetic apparatus is sensitive to heat stress (Berry and Björkman 1980). Integrity of the thylakoid membrane is strongly affected by heat stress (Pfeiffer and Krupinska 2005) and disturbs photosystems I and II (PSI and PSII) and the oxygenevolving complex (OEC), thus affecting phosphorylation (Dias and Lidon 2009; Rexroth *et al.* 2011). Heat stress causes ultrastructural changes such as expansion of the matrix zone, swelling and loosening of the lamella in the chloroplast. Heat stress may unstack grana and induce the formation of inverted cylindrical micelles (Zhang *et al.* 2014). Heat stress induced Reactive Oxygen Species (ROS) production across the thylakoid membrane causes peroxidation of membrane lipids, which accelerates the denaturation of thylakoid proteins thus intensifying electron leakage from membranes (Xu *et al.*, 2006).

2.3. Effect of Heat Stress on Cyclic and Noncyclic Electron Flow

Cyclic flow of electrons around PSI is triggered due to high temperature induced heat stress (Schrader *et al.* 2004). On the other hand, noncyclic electron transport is reduced, which significantly reduces NADPH (Sharkey and Schrader 2006) as electrons are diverted from the NADPH pool to the plastoquinone pool (Bukhov *et al.* 2005). This diversion may help to protect PSII from heat-induced damage by increasing zeaxanthin synthesis and reducing the size of light-harvesting antennae present on PSII (Sharkey and Schrader 2006).

2.4. Heat Shock Proteins (HSPs)

Heat shock proteins (Hsps) are a group of specific proteins which are produced primarily in response to heat shock in almost all biological systems. Under supraoptimal temperature, there is a dramatic change in protein synthesis in living organisms, with reduction in production of most proteins as well as the induction of HSPs. HSPs are molecular chaperones, which function in protein folding and assembly, protein intracellular localization and secretion, and degradation of



misfolded and truncated proteins. Heat shock factors (Hsfs) are the transcriptional activators of Hsps. Both Hsps and Hsfs are involved in response to various abiotic stresses such as heat, drought, salinity, and cold.

3. Effect of High Temperature Stress on Rice Productivity

Rice is highly sensitive to heat stress coinciding with the critical flowering stage (Jagadish *et al.*, 2010a, b). Exposure to short periods of heat stress coinciding with flowering have resulted in significant yield losses in India (Krishnan *et al.*, 2007), China (Tian *et al.*, 2010) and Japan (Hasegawa *et al.*, 2009). It has also been projected for other tropical and subtropical rice growing regions with such similar impacts on rice yield (Teixeira *et al.*, 2013). Heat stress during anthesis leads to irreversible reduction in spikelet fertility mainly due to a negative impact on sensitive physiological processes such as anther dehiscence and pollination, pollen germination on the stigma, pollen tube growth and/or early fertilization events (Jagadish *et al.*, 2010b, 2015).

3.1. Pollen Germination

Rice anthers dehisce at the time of floret opening and most of the florets, which are adichogamous, are self-pollinated. At the time of floret opening, it is the swelling of pollen grains that drive anther dehiscence (Matsui *et al.*, 1999). Pollination is very sensitive to temperature, low temperatures at the booting stage impede pollen growth (Shimazaki *et al.*, 1964), whereas high temperatures at the time of flowering inhibit the swelling of pollen grains (Matsui *et al.*, 2000) thereby affecting anther dehiscence. High (>35°C) and low (<20°C) temperatures during flowering stage can result in poor pollination and loss of yield (Hori *et al.*, 1992).

Changes are observed in some of the traits of reproductive organs such as increased anther pore size and reduced stigma length, and pollen number, and anther protein expression due to high temperature (Jagadish *et al.*, 2010). Variation in spikelet fertility was highly correlated with the proportion of spikelets with more than 20 germinated pollen grains on the stigma. The analysis of anther protein expression by a 2D-gel electrophoresis suggested that there were about 46 protein spots changing in abundance, of which 13 differentially expressed in both tolerant and susceptible genotypes. In the tolerant cv. N22, there was an upregulation of a cold and a heat shock protein, probably contributing to the heat tolerance.

3.2. Spikelet Sterility

Rice floral development is very sensitive to high temperatures. The susceptibility to high-temperature-induced floret sterility is highest at flowering stage, followed by booting stage (Satake and Yoshida, 1978). High temperature induced sterility varies with the cultivars: temperature above 35°C during anthesis can result in 90%



floral sterility in several rice cultivars (De Datta, 1981). Several controlled growth chamber studies clearly show high-temperature induced sterility, but probably it was Satake and Yoshida (1978) provided the most complete and precise information using the phytotron facilities at the IRRI (Philippines) and showed that anthesis was the most sensitive stage to high temperature in three indica (tropical) rice selections (IR747B2-6, N22 and BKN6624-46-2). The flowering of spikelets, immediately before or after high temperature, was not affected. Increase in temperature from 35 to 41oC as well as the duration of temperature treatment increased the percentage sterility. The night temperatures between 21 and 30oC did not affect spikelet fertility, but a night temperature of 33oC decreased fertility. Jagadish et al., 2007 observed that the spikelet temperature of 33.7°C even for an hour at anthesis induced sterility. But, temperatures of 38 and 41°C at an hour before or after anthesis do not affect spikelet fertility (Yoshida et al., 1981). Exposure to high temperature (centered on the time of peak anthesis) and duration (more than 2h) reduces spikelet fertility and genotypic ranking is highly correlated, suggesting a consistent and reproducible response of spikelet fertility to temperature (Jagadish et al., 2008).

To minimize heat stress damage in the field, three mechanisms are identified viz., heat escape (time of day of flowering (Julia and Dingkuhn 2012)), especially early morning flowering (Ishimaru *et al.*, 2010; Hirabayashi *et al.*, 2014), heat avoidance through transpiration cooling (Julia and Dingkuhn, 2013) and heat tolerance through resilient reproductive processes (Jagadish *et al.*, 2010).

Solutions to overcome current challenges faced with increasing temperatureinduced yield losses have advanced significantly, but examining the complex issues surrounding grain quality losses continues to be a major challenge. Additional challenges that could emerge with the transition from fully flooded rice cultivation to water-saving technologies need greater emphasis to ensure that the advantage gained under fully flooded conditions facilitates the transition with minimum damage under a future warmer and drier climate. To ensure sustained adoption of water-saving technologies under future hotter climates, rice cultivars with enhanced tolerance of heat and combined heat and drought stress during the floral meristem stage will be crucial to complement the progress achieved in overcoming the damage across other sensitive developmental stages such as flowering.

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Theme: IV

Modern technologies and innovative approaches to manipulate rice- photosynthesis under changing climate

-NRRI

Omics Technology in Agricultural Research

Gaurav Kumar

1. Introduction

New technologies that permit exact and accurate monitoring of hundreds, or thousands, of macro- and micro molecules allow functional monitoring of multiple key cellular pathways. The new and advanced technologies or methods that can be employed for measuring families of cellular molecules, such as DNA, RNA, proteins and intermediary metabolites have been termed as"-omic"technologies. The terms 'Ome' and 'Omics' are the derivations of the suffix -ome, which has been appended to a variety of previously existing biological terms like genome, proteome, transcriptome and metabolome. These tools help us to obtain complete assessments of the functional activity of biochemical pathways as well as structural genetic (sequence) differences among individuals and species. These tools and techniques are quite adequate in characterizing most members of a family of molecules in a single analysis. The basic aspect of these approaches is that a complex system can be understood more thoroughly with a holistic manner. Omic strategies help in discovery of biomarkers as they explore multiple molecules simultaneously. Omics enables a systems biology approach toward understanding the complex interactions between genes, proteins, and metabolites within the resulting phenotype. This integrated approach relies heavily on chemical-analytical methods, bioinformatics, and

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computational analysis and many disciplines of biology and have much potential and many applications in the field of medical, agriculture, biotechnology etc (Baginsky *et al.*, 2009). In last two decades wide application of omics technology in the agricultural research has played a vital role in enhancing the quality and nutritional composition of food crops; increasing agricultural production for food, feed and energy; playing an important role in crop protection; and significantly affecting the basic and applied research in important agricultural crops. The consistency and predictability in plant breeding has significantly improved due to the specific and precise use of genomics, proteomics, transcriptomics and metabolomics. Those not only reduces the time and expense of producing better quality food crops but also accelerate the production of multiple biotic or abiotic stress resistant/tolerant crops, without affecting its nutritional quality.

2. Classification of "Omics"

The four major 'Omics' technologies include genomics, transcriptomics, proteomics and metabolomics which are currently being used significantly in the scientific world. They are being developed with a goal of comprehensive and accurate detection of genes (genomics), mRNA (transcriptomics), proteins (proteomics) and metabolites (metabolomics) in a specific biological sample in a non-targeted and non-biased manner (Table-1)(fig 1). Other related and emerging omic technologies are foodomics, Lipidomics, Epigenomics, Immunomics, and Phenomics etc.

Table	1.	Summary	of	omic	technologies	
Technology		Description		What you le	arn	
Genomics		High throughput D of genomic DNA	High throughput DNA sequencing of genomic DNA		Which genes are present in an organism, and which alleles	
Transcriptomics		High throughput so RNA populations	equencing of	Which genes a tissue, cell type, environmentalstin	re transcribed ina or inresponse to nuli	
Proteomics Identification of proteins in a W protein population using mass spectrometry of oligopeptides		Which proteins are	Which proteins are expressed			
Metabolomics		Identification of m Cells	etabolites in	Which biochen produced, allo biochemicalpathw	nical productsare winginference of ⁄ays	



Fig. 1 The Central Dogma and the interacting "ome" includes the study of genome, proteome, transcriptome and metabolome

2.1. Genomics

The study of whole genomes of organisms, and incorporates elements from genetics is called genomics. The whole hereditary information of an organism is genome and is encoded in the DNA (or, for some viruses, RNA). Genome includes both genes and the non-coding sequences of the DNA. More precisely, the genome of an organism is a complete DNA sequence of one set of chromosomes and genomics is the study of an organism's genome. Genomics uses a combination of recombinant DNA, DNA sequencing methods, and bioinformatics to sequence, assemble and analyze the structure and function of genomes (Thompson *et al.*, 2016). This emerging field of molecular biology with advance novel technologies is exploited in order to understand the complex, biological function of the genome. A plant's



domestication and breeding history are recorded in its genome. The completion of the sequence of the first plant genome, Arabidopsis thaliana, helps in the post genomic era in plant research. A. thaliana is reported to have 25498 genes (The Arabidopsis Genome Initiative, 2000) and these genomes were completed along with the rice genome in the early 2000s which is estimated about 40,000 to 60,000 genes (plants. ensembl.org/Oryza_sativa/Info/Annotation) Genomics provides knowledge-based approaches for crop plant biotechnology, enabling precise and controllable methods for molecular breeding and marker-assisted selection, accelerating the development of new crop varieties. However, time is not the only advantage as new attributes not imagined before the omics era can be introduced into plants, such as the production of bio-pharmaceuticals and industrial compounds. Gene expression studies, identify functional gene products that give rise to the phenotype, provides which is information that can be used for plant improvements. By adding a specific gene or genes to a plant, or knocking down a gene with RNAi, the desirable phenotype can be produced more quickly than through traditional plant breeding

To start with genomics, sequencing is considered to be the obligatory procedure for the identification of known as well as unspecified variants in the genomic DNA. Next-generation DNA sequencing (NGS) has a huge impact on genomic studies. Recently, the most widely used platforms have been Roche/454 Life Science, Applied Biosystems SOLiD and Illumina Genome Analyzer. The most frequently used and high-throughput techniques used for functional genomics analysis are GTG banding technique, microarray-based comparative genomic hybridization (aCGH), fluorescent in situ hybridization (FISH) and Next-Generation Sequencing (NGS) (Debnath *et al.*, 2010).

2.2. Transcriptomics

Transcriptomics refers to the comprehensive scanning of all the currently known genes that are transcribed into RNA molecules from genome of an organism. Study of the transcriptome includes the analysis of complete set of RNA transcripts that are produced by the genome, under specific circumstances or in a specific cell using high-throughput methods, such as microarray analysis or RNA sequencing. Comparison of transcriptomes allows us to identify the genes which are differentially expressed in diverse cell populations, or in response to different treatments. For example, by comparing such transcriptional analyses from plants exposed to different environmental conditions it is possible to quickly identify all genes involved in the adaptive process (Dunwell *et al.*, 2001). All genes and most proteins can be regarded as instruments for making up the biochemical composition and there by the physiological identity of an organism.

Transcriptomics is now easy, cheap and fast enough and can be done with great precision. The RNA analysis was once limited to tracking individual transcripts

by Northern blots or quantitative PCR, but high throughput transcriptomics is now possible with microarrays, which detect nucleic acid by hybridization to probes on microchips. Microarrays are particularly useful for analyzing large number of transcriptomes with known sequences, so they can't be used for discovery. Even though microarray technology continues to advance (Fig 2) Transcriptomics has expanded dramatically in the past few years because of developments in RNA sequencing (RNA-seq) technique. The development of high-throughput strategies is becoming increasingly important in biology because they permit to monitor the expression levels of thousands of genes in only one experiment. To interpret the biology of these genetic profiles, these data must be analysed in the context of the corresponding proteins coded. This means retrieving information about their biological, biochemical or molecular function (Horgan *et al.*, 2011). Thus, the development of computational tools to compare and analyze these expression profiles is very much necessary for biological interpretation.

With recent advances in sequencing technologies, genome assemblies of multiple $C_4 C_4$ species including maize, sorghum and new C_4 model species, foxtail millet (Setaria italica), are currently available, providing a good opportunity to dissect the C_4 pathway using system biology approaches. To date, several transcriptomics and proteomics studies have provided insight into C_4 gene expression and protein accumulation by comparative analysis of bundle sheath (BS) and mesophyll (ME) cells in maize, green foxtail (Setaria viridis), and rice. Transcriptional profiling along a leaf development gradient in maize, between maize and rice, between both distantly and closely related C_3 and C_4 species has been studied (Lieben *et al.*, 2017). Hundreds of differentially accumulated genes and proteins were identified and functionally characterized, which will not only provide important insights into the differential gene regulation between C_3 and C_4 species but also provide a good genetic resource for establishing C_4 pathways in C_3 crops.



Fig. 2 Analytical technique showing Process flow for gene expression microarray experiment

2.2.1. Identification of Photosynthesis-Associated C_4 Candidate Genes through Comparative Leaf Gradient Transcriptome in Multiple Lineages of C_3 and C_4 Species

Extreme climate changes, decreasing availability of water and energy resources, and competitions between food-crops for bio-fuels and food could worsen the situation. One of the most promising solutions is to introduce the C_4 photosynthetic pathways into C_3 crops such as rice and soybeans, to improve their water, radiation, and nitrogen use efficiency resulting in higher yields than present day C_3 crops.

Recently, novel cell type-specific cis-regulatory elements and candidate transcription factors of C₄ photosynthesis have been identified, by comparing sets of leaf gradient transcriptome data from maize and rice. Still it is less known about the downstream regulatory networks of genes and protein interactions responsible for the fundamental anatomical features in both C4 and C3 species, as well as the mechanisms controlling the expression and function of well characterized C_4 genes. Systems biology analysis of multiple lineages of C_3 and C_4 species and comparative studies across species could provide great promise for identifying unknown genes that control many, yet unknown, C_{A} functions. To better understand the function of C4 photosynthesis, and to identify candidate genes that are associated with the C₄ pathways, a comparative transcription network analysis was conducted on leaf developmental gradients of three C4 species including maize, green foxtail and sorghum and one C₃ species, rice. By combining the methods of gene co-expression and differentially co-expression networks, identified a total of 128 C₄ specific genes. Besides the classic C4 shuttle genes, a new set of genes associated with light reaction, starch and sucrose metabolism, metabolites transportation, as well as transcription regulation, were identified as involved in C_4 photosynthesis.

The salient findings from this novel technique comparative leaf gradient transcriptome showed that the photorespiration genes were co-expressed in rice but they were not co-expressed in C_4 species. Classical C_4 genes, e.g., carbonic anhydrase (CA), phosphoenolpyruvate carboxylase (PEPC), NADP-malate dehydrogenase (NADP-MDH), NADP-malic enzyme (NADP-ME), pyruvate orthophosphate dikinase (PPDK) and PPDK regulatory protein (PPDK-RP), showed an increasing expression profile from the base to tip along the leaf. Except CA the expression of PEPC and PPDK was much lower in rice than in the other three C_4 species. Classical C_4 genes either decreased in their expression level or changed their expression pattern in rice. Thus, the characteristics of classical C_4 genes can be used as a criterion to define other C_4 -related candidate genes (Ding *et al.*, 2015). Overall, these complementary approaches will increase our understanding of the C_4 pathway and facilitate attempts to engineer it into C_3 species. Although transcriptomics studies can reveal the relative amounts of different mRNAs in the cell, levels of mRNA are not directly proportional to the expression level of the

proteins they code for. The number of protein molecules synthesized using a given mRNA molecule as a template is highly dependent on translation-initiation features of the mRNA sequence.

2.3. Proteomics

Improvements in analytical technology and continuous generation of genomics and transcriptomics information strengthen the area of proteomics in many different aspects. Since proteins serve as important components of major signaling and biochemical pathways, studies at protein levels are essential to reveal molecular mechanisms underlying growth, development and interactions with the environment. Proteomics is the application of evolving technologies to analyze gene products, proteins, on a large scale. Proteomics has rapidly emerged as an exciting new field of research, one that complements rather than replaces genomics. It is an integrated approach to study the proteins and their biological functions and processes associated with them, such as hormone production, immune-system responses etc (Debnath *et al.*, 2010). It facilitates the study of protein structure and protein – protein interactions. Hence today this branch of science is becoming a major tool in biomedical, drug development research and basic as well as agricultural science.

Since proteins play a central role in the life of an organism, proteomics is beneficial in discovery of biomarkers, such as markers that indicate a particular deviation from normal. In plants also, proteins are every where and are responsible for many cell functions. Through proteomics, it can be determined, whether expression of mRNA results in protein synthesis to further validate gene function. The hundreds thousands of distinct proteins in plants play key functional roles for the texture, yield, flavor, and nutritional value of virtually all food products (Jeanette et al., 2015). Through protein expression profiling, proteins can be identified at a specific time as a result of expression to a stimulus such as disease and insect infestation or temperature and drought, further elucidating the function of particular proteins. Comparative proteomics can determine the molecular mechanisms for susceptibility or resistance to stress. The molecular biology revolution and the advent of genomic and proteomic technologies are facilitating rapid advances in our understanding of the molecular details of cell and tissue function. Older methods involved isolation of proteins by 2D electrophoresis, but more recent approaches employ more high-throughput methods (Fig 3) like Matrix-assisted laser desorption ionization (MALDI)/MS, Protein microarrays or chips, Isotope-coded affinity tag (ICAT) labeling, stable isotope labeling with amino acids in cell culture (SILAC) and isobaric tag for relative and absolute quantitation (iTRAQ) techniques have recently developed for quantitative proteomics (Baginsky et al., 2009). X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy are two major high-throughput techniques that provide three-dimensional (3D) structure of protein that might be helpful to understand its biological function (Horgan et al., 2011).





Fig. 3 Analytical techniques showing simplified schematic work flow for proteomics experiments

2.4. Metabolomics

Many of the proteins in a cell are enzymes which catalyze reactions leading to a large set of products. The set of primary and secondary products (metabolites) produced from the enzymes of metabolism in the cell at any instance is referred to the metabolome. Study of techniques for separating metabolites from a cell, based on charge, molecular weight and other characteristics is known as metabolomics. In general, metabolomics is the comprehensive study of the metabolome, the collection of biochemicals (or small molecules) present in cells, tissue, and body fluids. Following the rapid developments in genome, transcriptome and proteome technology, there is a growing interest in metabolome research. The study of metabolism at the global or "omics" level is a new but rapidly growing field that has the potential to have an impact upon agricultural research in general and rice physiology in particular, (Jeanette et al., 2015). The metabolome is dynamic and highly influenced by environmental and internal conditions. Hence, the metabolic condition indicates about what has been encoded by the genome and how it is modified by environmental factors. The real-time monitoring of metabolic networks allow us to understand the changes resulting from biotic or abiotic stress, which can aid in the basic understanding of systems biology and development of improved crop varieties. Metabolic profiling provides real time information of what is happening in the cell, for example, during grain development, identifying key compounds important for imparting aroma and taste (Kumar et al., 2017). The constant watch on metabolite dynamic patterns can help in quality improvement for nutrition and plant health. The recent rapid development of a range of analytical

platforms, including GC, HPLC, UPLC, CE coupled to MS and NMR spectroscopy, could enable separation, detection, characterization and quantification of most of the metabolites involved in various metabolic pathways (Hong *et al.*, 2016)(Fig 4).



Fig. 4 Analytical techniques showing simplified schematic work flow for metabolomics study

3. Conclusion

Omics will not produce a vegetable or cereals, but it can provide potential health benefits to consumers, contribute to a stable food and energy supply, help in preserving and protecting the environment, benefit farmers, and help eliminate world hunger. Omics has enhanced our understanding of pesticide bio-degradation and the mechanisms of pesticide resistance and metabolism, leading to the development of more effective and safer pesticides and the identification of biomarkers to determine human and plant under adverse environmental exposures (Jeanette et al., 2015). The increased knowledge and insights gained from plant genomics are leading to unexpected discoveries and conceptual advances in understanding plant biology. Through the advances made by omics, large-scale collections of proteins (proteomes), interactions between proteins (interactomes), metabolites (metabolomes), and collections of observable characteristics (phenomes), are enabling a systems-biology approach for understanding plants from the single cell to the mature plant not only during development but importantly, under changing environmental conditions (Horgan et al., 2011). Omics has increased our ability to feed a hungry world, particularly populations that live in less than ideal agricultural regions. Scientists in omics research need to do their part by dispelling opinions

with facts. However the modification of biological organisms through any process can carry potential safety risks, raising issues that must be defined and addressed in a manner to satisfy consumers. To ensure bio-safety, scientific expertise must be applied to analyse the biotech crops, their progeny, and anticipated environmental interactions for a sound risk assessment, which ironically can be accomplished in part with omic studies. We can see that omics can revolutionize agricultural research in many exciting areas and fulfill the researcher's vision for the future of agriculture rice physiology. The omics pipeline is full of promise including more functional foods, such as tomatoes with high levels of flavonols to reduce health risks, foods with higher levels of phytosterols to reduce cholesterol, and plants for producing drugs to address specific health issues. Drought-tolerant maize, arsenictolerant plants, low-lignin trees for papermaking, longer bananas with a longer shelf life, plants that can fix ambient N2 and plants with increased bio-luminescence to provide lighting are all potentials.

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Manipulating Photorespiration to Increase Plant Productivity: Recent Advances and Perspectives for Crop Improvement

Awadhesh Kumar

1. Introduction

Enormous improvements in grain yield achieved in the past by green revolution seems to be inadequate to feed the exponentially increasing global population and further there is need for another quantum leap in agricultural productivity. As the world population races towards 10 billion, agricultural scientists are realizing that another "green revolution" is needed for boosting crop yields to meet demands for food (von Caemmerer, 2012). According to the World Food Summit held in 1996 at Rome, food security is defined as "when all people, at all times, have physical and economic access to sufficient, safe and nutritious food to meet their dietary needs and food preferences for an active and healthy life". The required increase in agricultural productivity by 2030 may be around~60% as compared with the levels what is the level in percentage of 2005 (Ort et al., 2015). However, with a burgeoning population, decreasing arable land, stagnation in agricultural production, the environmental changes due to global warming along with various biotic and abiotic stresses, it becomes an overwhelming task to ensure complete food and nutrient security. One fundamental component of plant productivity that has yet to be fully utilized is the potential for increasing yield by improvement in photosynthesis.

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2. Photosynthesis

Photosynthesis is the mechanism through which chloroplastic enzyme RuBisCO(Ribulose-1,5-bisphosphate carboxylase/oxygenase) captures CO_2 and transfers it into sugar, thereby creating biomass. In C_3 plants, the efficiency of carbon fixation is not optimal in certain environmental condition where part of fixed carbon is lost through the oxygenation activity of RuBisCO. The balance between these two activities depends mainly on the CO_2/O_2 ratio in the leaves, which may change following the plants reaction to certain environmental condition. Carboxylase reaction forms the 2 molecules of PGA (phosphoglycerate) where Oxygenase reaction forms the 1 molecule of PGA and 1 molecule of phosphoglycolate [Fig.1]. Metabolism of phosphoglycolate under photorespiration takes several exhaustive and energy consuming steps for the formation of PGA.



Fig. 1 Schematic representations of RuBisCO photosynthesis and photorespiration. At the catalytic site of RuBisCO, competition occurs between CO_2 and O_2 for carboxylase and oxygenase activity. Metabolism of phosphoglycolate takes several steps for the formation of PGA and the metabolism process called photorespiration.

3. Photorespiration

RuBisCO evolved 3 billion years ago when the concentration of CO_2 in atmosphere was higher than now and concentration of O_2 was almost zero and oxygenation activity was very low. Now the atmospheric oxygen concentration is much higher than 3 billion years ago which supports the RuBisCO oxygenase activity and this further increases when the temperature rises (Ehleringer, 2001) [Fig.2]. At current CO_2 level, photorespiration can reduce photosynthesis by 40% at high



temperature in C_3 plants (Ehleringer *et al.*, 1991). Metabolisms of phosphoglycolate into PGA performs in three different compartments (chloroplast, peroxisome and mitochondria) with 16 enzymes and more than 6 translocaters. So the whole process of photorespiration decreases the photosynthetic efficiency of C_3 plants (Douce and Neuburger, 1999). Each round of photorespiration generates one molecule each of H_2O_2 and NH_3 which are cytotoxic and require energy for their metabolism.



Fig. 2 Photorespiration in future agricultural production systems. Impact of climate change and the increased demands for food and fuel on photorespiratory losses.

3.1. Beneficial effects of Photorespiration

While photorespiration seems to be a wasteful process regarding carbon and energy losses, it has important role in plant as it recovers 75% of the carbon from phosphoglycolate as well as efficiently removes potent inhibitors of photosynthesis. The current knowledge suggests that photorespiration is important for plant survival, and may provide reducing power for nitrate (NO₃) assimilation (Kozaki *et al.*, 1996; Rachmilevitch., 2004). Moreover, photorespiration dissipates excess photochemical energy under high light intensities, thus protecting the chloroplast from over-reduction (Wingler *et al.*, 2000; Kozaki *et al.*, 1996). Under stress conditions (drought, salinity, cold, high light, heat), an excess of NADPH may be produced and leads to increase of reactive oxygen species (ROS) where photorespiration can act as a sink for this excess reducing power (Peterhänsel *et al.*, 2010).

4. Different Check Points Targeted to Improve Photosynthesis

The projected increase in yields of C_3 crops as a result of increasing atmospheric CO_2 concentrations is much less than anticipated. Thus, there is an urgent need to increase crop productivity beyond existing yield potentials to address the challenge of food security. One of the domains of plant biology that promises hope in overcoming this problem is the study of C_3 photosynthesis. There are at least five different check points present in leaf of C_3 plant which are considered for improvement of photosynthesis by overcoming these bottlenecks in Calvin cycle (Singh *et al.*, 2014) [Fig. 3].



Fig.3 Different check points targeted to improve photosynthesis. (1) RuBisCO, (2) RuBisCO activase (RCA), (3) Regeneration of Ribulose-1,5- bisphosphate (RuBP), (4) CO_2 transport across mesophyll cell membrane and (5) Photorespiration

5. Engineering Efforts to Improve Photosynthesis:

There is an urgent need to improve agricultural productivity to secure future food and biofuel supply. To increase the yield of photosynthetic CO_2 fixation, different strategies are suggested which can be at pursued to achieve this goal [Fig. 4]. These strategies fall into four categories: (i) Improvement of the catalytic properties of RuBisCO (ii) Improvement in the working conditions of RuBisCO through CO_2 concentrating mechanisms (CCM) (iii) Engineering Synthetic photorespiration bypasses (iv)Engineering of the synthetic CO_2 fixation pathways.



Fig.4 Possible engineering efforts for improvement in the photosynthetic CO₂ fixation

6. Strategy for Enhancing CO_2 Fixation and Minimizing Photorespiratory Effect

While most efforts are aimed at generating plants with reduced photorespiratory rates, the eventual performance of these plants in the field (especially under stress conditions) needs also to be considered. Promising results have been shown in model plants engineered through photorespiratory pathway, but the transfer of these manipulations to major crops and the demonstration of benefits under field conditions are still lacking (Kebeish *et al.*, 2007; Timm *et al.*, 2012a). Following different approaches have been used to manipulate photorespiration for crop improvement:

- i. Screening for plants with naturally reduced rates of photorespiration.
- ii. Enhancing photorespiratory CO₂ scavenging capability.
- iii. Introduction of C_4 metabolism into C_3 species.
- iv. Introduction of CO₂ concentrating mechanisms into chloroplasts.
- v. RuBisCO engineering and screening for natural variation.
- vi. Introduction of Photorespiratory bypass to enhance CO₂ concentration.

6.1. Screening for Plants with Naturally Reduced Rates of Photorespiration

Mutagenized barley and Arabidopsis with altered phenotype showed reduced photorespiration under normal air conditions (Peterhänsel *et al.*, 2010) whereas mutagenized tobacco plants identified with higher yield at lower CO_2 concentrations (Medrano *et al.*, 1995). The mutants obtained have shown poor performance under normal air conditions associated with different stress symptoms. This approach permitted the identification of the genes encoding core enzymes of the photorespiratory cycle (Timm and Bauwe, 2013). Natural variants of tobacco with reduced rates of photorespiration was found resulting higher yields (Zelitch and Day, 1973). Some wheat and soybean lines were identified with high photosynthetic but low photorespiratory rates and their yield was constantly high. Highly productive cultivars have shown high rates of photosynthesis accompanied by high rates of photorespiration (Aliyev, 2012). These findings suggested that genotypes with high photosynthetic but low photorespiratory rates are inconsistent.

6.2. Enhancing Photorespiratory CO₂ Scavenging Capability

Through the decarboxylation step of photorespiration released CO_2 in mitochondria is not completely lost for plant. The released CO_2 can be refixed while passing through the chloroplast by some plants through photorespiratory CO_2 scavenging. Chloroplast forms a barrier that covers cell wall space to trap photorespiratory CO_2 . This effect can be enhanced by a tight association between mitochondria and chloroplast (Busch *et al.*, 2013). Lesser chloroplast cover and mitochondria that are not in close contact with the chloroplast result in a lower capacity to scavenge



photorespiratory CO_2 as in rice [Fig.5]. But, modification of cell anatomy has little impact on cell metabolism and installation of this anatomy in a plant needs better understanding of organelle movement and partitioning.



Fig. 5 The effect of cover and positioning on photorespiratory CO_2 scavenging (A) When chloroplasts (c) cover a large portion of the cell wall space adjacent to the intercellular air space, they provide a barrier for the photorespiratory CO_2 released by the mitochondria (m), which can then be re-assimilated in the chloroplast. Tight associations between mitochondria and chloroplasts add to this effect. In addition, a high chloroplast cover reduces the resistance for CO_2 entering the chloroplast from the outside of the cell. Both processes increase the CO_2 concentration in the chloroplast and thereby reduce photorespiration. (B) Low chloroplast cover and/or mitochondria that are not in close contact with the chloroplasts result in a lower capacity to scavenge photorespiratory CO_2 .

6.3. Introduction of C₄ Metabolism into C₃ Species

The C₄ photosynthesis greatly reduces photorespiration by concentrating CO₂ near RuBisCO. C₄ leaves have Kranz leaf anatomy (two distinct layers of photosynthetic tissues) where mesophyll cells are in contact with atmospheric CO₂ through intercellular air spaces and bundle sheath cells with cell walls are less permeable to CO₂. HCO3 is converted into oxaloacetate in the mesophyll cells via phosphoenolpyruvate carboxylase, which is then converted to a more stable four carbon organic acid (malic or aspartic acid) which then diffuses to the bundle sheath cells. Here the C₄ acid is decarboxylated, releasing CO₂ near RuBisCO [Fig. 6].



Fig.6 Difference between CO₂ metabolism into C₃ and C₄ species (A) C₃ photosynthesis fixes atmospheric CO₂ into C₃ acids with RuBisCO in single cells. (B) The rice section shows vascular bundles with few chloroplasts and large numbers of mesophyll cells between the vascular bundles typical for C₃ species. (C) Two-cell C₄ photosynthesis requires spatial separation of fixation of atmospheric CO₂ into C₄ acids and the donation of CO₂ from these C₄ acids to RuBisCO. (D) The sorghum leaf section shows chloroplasts in bundle sheath and only two or three mesophyll cells in between the vascular tissue typical of a C₄ species.

With the higher efficiency of the C_4 photosynthetic mechanism under current atmospheric CO₂ concentration, efforts are underway to install C₄ photosynthesis in C₃ plants such as rice (the International C₄ Rice Consortium, http://c4rice.irri. org/) [Fig.7] and other crops. Over expression of seduheptulase bisphosphtase (SBPase) in tobacco and rice showed improved photosynthesis and accumulation of biomass (Tamoi *et al.*, 2006). Expression of cyanobacterial carbonic anhydrase in tobacco increases photosynthesis rate (Lieman-Hurwitz *et al.*, 2003). Expressing PEPC from Corynebacterium glutamicum and NADP-ME from Flaveria pringlei in potato showed enhancement of CO₂ assimilation at higher temperature and also increased respiration under dark condition (Lipka *et al.*, 1999). Expression of C₄ specific PEPC and PPDK from maize, NADP-MDH from Sorghum and C₃ specific NADP-ME from rice show slightly higher CO₂ assimilation rate than wild type plants (Taniguchi *et al.*, 2008). Engineering of NADP-ME subtype photosynthesis

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into indica rice was attempted by the C_4 rice consortium. While the number of genes necessary for the main enzymatic reactions and transporters involved in C_4 photosynthesis is relatively small, the introduction of C_4 photosynthesis into C_3 crops will also require major changes in leaf anatomy (von Caemmerer *et al.*, 2012). All these approaches showed limited success to get higher biomass in C_3 plants having manipulated Calvin cycle using heterologous genes, but each of these approaches give new information for the behaviour of the foreign gene.

Fig.7 Biochemical pathway of NADP-ME subtype of C4 photosynthesis engineered into



indica rice by the C_4 rice consortium.

PEPC does the first carboxylation in the mesophyll cell producing oxaloacetate which is further converted to malate by MDH. This C_4 acid is transported from mesophyll cell to the bundle sheath cell where it is decarboxylated by NADP-ME to pyruvate and CO_2 is released to RuBisCO to carry out the Calvin cycle reactions. In C_4 rice, RuBisCO should be expressed in bundle sheath cell and hence the increased CO_2 levels at its site will reduce its oxygenation activity subsequently reducing the photorespiration.

6.4. Introduction of CO, Concentrating Mechanisms into Chloroplasts

To reduce photorespiration in plant, introduction of cyanobacterial CCM into the chloroplasts is the another strategy to reduce oxygenation (Price *et al.*, 2013). Cyanobacteria concentrates CO_2 inside the proteinaceous microcompartment called carboxysome and suppress the oxygenation reaction of RuBisCO. An outer shell composed of β -carboxysome enclose RuBisCO and carbonic anhydrase maintaining high CO_2 concentration inside the microcompartment and increasing the catalytic efficiency of the carboxylation reaction of the enzyme [Fig. 8]. Engineering of CCM into chloroplast of higher plant to express a functional cyanobacterial form of RuBisCO together with proteins involved in the enzyme's assembly resulted into reduced photorespiration in tobacco plants (Lin *et al.*, 2014). However, the engineered plants were able to survive only at high CO₂ concentration.





Cyanobacteria and algae have developed independent analogous mechanism for the concentration of CO₂ in the vicinity of RuBisCO.

6.5. Rubisco Engineering and Screening for Natural Variation

In addition to oxygenase activity, RuBisCO has low kcat value for CO_2 that obliges plants to produce very high amounts of the enzyme to sustain adequate photosynthesis, needs a large nitrogen investment (Zhu *et al.*, 2007). Transformation of both the nuclear and chloroplast genomes of the same plant is thus required to make a more efficient enzyme for the endogenous one. Challenge for replacing the



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plant endogenous RuBisCO with a more efficient one is that the large subunit of the enzyme is encoded by a single chloroplastic gene and the small one by several nuclear genes. The active sites of RuBisCO is encoded by its large subunit, changing only the large subunit may improve enzyme efficiency. Expressing the thermostable version of RuBisCO activase lead to increase in the biomass because the Calvin cycle is sensitive to higher temperature. So making some RuBisCO intermediate capable to increase the capacity of enzyme (Kurek *et al.*, 2007). using transplastomic tobacco lines expressing native and mutated RuBisCO large subunits from Flaveria pringlei (C₃), Flaveria floridana (C₃-C₄), and Flaveria bidentis (C₄), Whitney *et al.*, (2011b) revealed that Met-309-IIe substitutions in the large subunit act as a catalytic switch between C₄ and C₃ catalysis.

6.6. Introduction of Photorespiratory Bypass to Enhance CO_2 Concentration

Apart from trying the strategy to reduce the photorespiratory rates, another approach is to install alternative energetically less expensive routes for the recycling of phosphoglycolate (2PG). Till date, six Photorespiratory bypass experiments have been successfully engineered in plants.

- i. Introduction of E. coli glycolate catabolic pathway into A. thaliana where glycolate was converted to glycerate in chloroplast (Kebeish *et al.*, 2007).
- ii. Introduction of partial bypass ofglycolate catabolic pathway into potato (Solanum tuberosum) by expressing the E. coli glycolate dehydrogenase polyprotein (Nölke *et al.*, 2014).
- iii. Glycolate catabolic pathway genes from E.coli were introduced into Camelina sativa chloroplast (Dalal *et al.*, 2015).
- iv. Introduction of a complete glycolate catabolic cycle into A. thaliana where 2PG oxidized to CO_2 in the chloroplast (Maier *et al.*, 2012).
- v. Introduction of E. coli glyoxylate carboligase and hydroxypyruvate isomerase into tobacco for the conversion of glyoxylate into hydroxypyruvate directly in the peroxisome (Carvalho *et al.*, 2011).
- vi. Synthetic CO_2 fixing pathway (the hydroxypropionate bi-cycle) engineered in a cyanobacterium worked both as a photorespiratory bypass and as an additional CO_2 fixing pathway (Shih *et al.*, 2014).

In experiment i-iii, glycolate was converted to glycerate directly in the chloroplast by introducing the E. coli glycolate catabolic pathway, thus increasing concentration of CO_2 nearby RuBisCO (Kebeish et al., 2007; Nölke et al., 2014; Dalal et al., 2015). The 4th experiment was related to introduction of a complete glycolate catabolic cycle that oxidized 2PG to CO_2 in the chloroplast (Maier et al., 2012). Experiment i-iii resulted in an improved energy balance, while 'Maier' bypass had higher energetic costs compared with the standard photorespiratory

cycle (Peterhänsel et al., 2013b). Carvalho et al., 2011 (experiment v) engineered the photorespiration pathway by introducing the E. coli enzymes glyoxylate carboligase and hydroxypyruvate isomerase into tobacco for the conversion of glyoxylate into hydroxypyruvate directly in the peroxisome. In a recent work (experiment vi), a synthetic pathway that worked both as a photorespiratory bypass and as an additional CO_2 fixing pathway, the hydroxypropionate bi-cycle, was successfully engineered in a cyanobacterium (Shih et al., 2014).

6.6.2. Introduction of *E. coli* glycolate catabolic pathway into *A. thaliana* where glycolate was converted to glycerate in chloroplast.

Using step-wise nuclear transformation with five chloroplast-targeted bacterial glycolate catabolic genes, plants were generated where glycolate is converted directly to glycerate. This reduces, but does not eliminate, flux of photorespiratory metabolites through peroxisomes and mitochondria [Fig.9]. Similar pattern of findings were also observed in plants transformed with the three subunits of glycolate dehydrogenase, but enhanced by introducing the complete bacterial glycolate catabolic pathway. Transgenic plants grew faster, produced more shoot and root biomass, contained more soluble sugars, reflecting reduced photorespiration and enhanced photosynthesis which correlated with an increased chloroplastic CO_2 concentration in the vicinity of RuBisCO carboxylase/ oxygenase.



Fig. 9 Glycolate catabolic pathway (red marked) of E. coli introduced in Arabidopsis plant chloroplast

RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose-1,5-bisphosphate; PGP, phosphogycolate phosphatase; GOX, glycolate oxidase; CAT, catalase; GGAT, glyoxylate/glutamate aminotransferase; GDC/SHMT, glycine decarboxylase/serine hydroxymethyl transferase; SGAT, serine/glyoxylate aminotransferase; HPR, hydroxypyruvate reductase; GK, glycerate kinase; GS, glutamine synthetase; GOGAT, glutamate/ oxoglutarate aminotransferase; Fdred, reduced ferredoxin; Fdox, oxidized ferredoxin; GDH, glycolate dehydrogenase; GCL, glyoxylate carboxyligase; P-glycerate; phosphoglycerate; TSR, tartronic semialdehyde reductase.

6.6.3. Introduction of Partial bypass of Glycolate Catabolic Pathway into Potato (Solanum tuberosum) by Expressing the E. coli Glycolate Dehydrogenase Polyprotein

A polyprotein (DEFp) comprising all three subunits (D, E and F) of E. coli glycolate dehydrogenase (GlcDH) was introduced into potato plant. The engineered polyprotein retained the functionality of the native GlcDH complex when expressed in E. coli and was able to complement mutants deficient for the D, E and F subunits. Transgenic plants accumulated DEFp in the plastids and the recombinant protein was active in planta resulted in reducing photorespiration and improving CO_2 uptake with a significant impact on carbon metabolism. Transgenic lines with the highest DEFp levels and GlcDH activity produced significantly higher levels of glucose (5.8-fold), fructose (3.8-fold), sucrose (1.6-fold), resulting in a substantial increase in shoot and leaf biomass. The higher carbohydrate levels produced in potato leaves were utilized by the sink capacity of the tubers, increasing the tuber yield by 2.3-fold.

6.6.4. Introduction of Glycolate Catabolic Pathway Genes from E.coli into Camelina Sativa chloroplast

Genes encoding three enzymes of the E. coli glycolate catabolic pathway were introduced in Camelina sativa. These enzymes compete for the photorespiratory substrate, glycolate and convert it to glycerate within the chloroplasts, resulting reduced photorespiration. As a by-product of the reaction, CO_2 is released in the chloroplast, which increases photosynthesis. Camelina plants were transformed with either partial bypass (GDH), or full bypass (GDH, GCL and TSR) genes. Expression of partial bypass increased seed yield by 50-57 %, while expression of full bypass increased seed yield by 57-73 %, with no loss in seed quality. The transgenic plants also showed increased vegetative biomass and faster development; they flowered, set seed and reached seed maturity about 1 week earlier than wild type (WT) plant. The increased growth of the bypass transgenics compared to WT was only observed

in ambient or low CO₂ conditions, but not in elevated CO₂ conditions.

6.6.5. Introduction of a Complete Glycolate Catabolic Cycle into A. thaliana where 2PG Oxidized to CO, in the Chloroplast

Photorespiratory pathway is considered futile because previously assimilated CO_2 is released in mitochondria. A lot of efforts has been made to reduce this CO_2 loss either by reducing fluxes via engineering RuBisCO or circumventing mitochondrial CO_2 release by the introduction of new enzyme activities. A complete glycolate catabolic cycle was introduced in the chloroplasts of A. thaliana comprising glycolate oxidase (GO), malate synthase (MS) and catalase (CAT). Transgenic lines bearing all three transgenic enzyme activities were identified and showed higher



GO, MS and CAT activity resulted in higher dry weight, higher photosynthetic rates compared to the WT [Fig10].

Fig. 10 The photorespiratory carbon and nitrogencycle of a C_3 -plant short-circuited by the novel glycolate catabolic pathway (red marked)

The transgenic enzymes introduced into A. thaliana chloroplasts are highlighted (green marked). DiT1: dicarboxylate translocator1; DiT2: dicarboxylate translocator2; CAT: catalase; GDC: glycine decarboxylase; GGAT: glutamate-

glyoxylate aminotransferase; GLYK: glyceratekinase; GO: glycolate oxidase; GOGAT: glutamate-oxoglutarate aminotransferase; GS: glutamine synthetase; HPR: hydroxypyruvate reductase; ME: NADP-malic enzyme; MS: malate synthase; PDH: pyruvate dehydrogenase; PGP: phosphoglycolate phosphatase; SGAT: serine-glutamate aminotransferase; SHMT: serine hydroxymethyl transferase.

6.6.6. Introduction of E. coli Glyoxylate Carboligase and Hydroxypyruvate Isomerase into Tobacco for the Conversion of Glyoxylate into Hydroxypyruvate Directly in the Peroxisome

The liberated ammonia during photorespiration is re-assimilated, but up to 25% of the carbon may be released into the atmosphere as CO_2 . Because of the loss of CO_2 and high energy costs, there has been considerable interest in attempts to decrease the flux through the cycle in C_3 plants. Transgenic tobacco plants contained the genes gcl and hyi from E. coli encoding glyoxylate carboligase and hydroxypyruvate isomerase, targeted to the peroxisomes. It was presumed that the two enzymes could work together and compete with the aminotransferases that converts glyoxylate to glycine, thus avoiding ammonia production in the photorespiratory nitrogen cycle. Tobacco plants have been generated that produce bacterial glyoxylate carboligase but not hydroxypyruvate isomerase. The transgenic plants exhibit a stress response when exposed to air, suggesting that some glyoxylate is diverted away from conversion to glycine in a deleterious short-circuit of the photorespiratory nitrogen cycle. This diversion in metabolism gave rise to increased concentrations of amino acids, in particular glutamine and asparagine in the leaves and a decrease of soluble sugars [Fig11].



Fig. 11 The Photorespiratory Nitrogen Cycle showing the alternative route through tartronic semialdehyde
The ammonia produced in the conversion of glycine to serine passes out of the mitochondrion and is reassimilated (green pathway). The CO_2 released in the mitochondrion escapes to the intercellular spaces. The red pathway represents the intended short-circuit in the photorespiratory cycle by the bacterial enzymes gcl and hyi. (Glu: Glutamate; Gln: Glutamine; 2OG: 2-oxoglutarate; OAA: oxaloacetate; TSA tartronic semialdehyde).

6.6.7. Synthetic CO₂ Fixing Pathway (the Hydroxypropionate bi-cycle) Engineered in a Cyanobacterium Worked Both as a Photorespiratory Bypass and as an Additional CO₂ Fixing Pathway.

A synthetic pathway that worked both as a photorespiratory bypass and as an additional CO_2 -fixing pathway, the hydroxypropionate bi-cycle, was successfully engineered in a cyanobacterium. Functions of all the sixintroduced enzymes was demonstrated. In comparison to the conventional C2 cycle, the synthetic bypass not only prevents the loss of NH₃ but also results in a net gain in carbon fixation rather than a net loss [Fig. 12].



Fig. 12 Engineering a synthetic cyclic photorespiratory bypass which also fixes bicarbonate.

Enzymes in white boxes are present in cyanobacteria and plants. The six additional enzymes required to establish this CO_2 fixing photorespiratory bypass are in colored boxes. One bicarbonate molecule is fixed while one glyoxylate is consumed to form pyruvate, which can be used for biosynthesis or to replenish the CB cycle. Acc: acetyl-CoA carboxylase; Mcr: malonyl-CoA reductuse; Pcs: propionyl-

CoA synthase; Mcl: malyl-CoA lyase; Mch: mesaconyl-C1-CoA hydratase; Mct: mesaconyl-CoA C1:C₄ CoA transferase; Meh: mesaconyl-C₄-CoA hydratase; Pgp: 2-phosphoglycolate phosphatase; GlcD: glycolate dehydrogenase, RuBisCO: ribulose-1,5-bisphosphate carboxylase/oxygenase.

7. Future Perspectives for Crop Improvement

Several approaches have been used for manipulation of photorespiration (mainly using model plants). Efforts should now initiate in major crops (like consortium working on the transformation of rice into a C₄ plant). As per results obtained by recent field trials, it would appear unlikely that crops with improved photorespiratory performance can be obtained by screening for natural genetic variation. It should rather be generated by means of genetic engineering. Transformation technique of chloroplast DNA for manipulation of the large subunit of RuBisCO is available only for a few crop species. This technique should be standardized for cereal species. Before tackling the genetic engineering of crop species, organisms for which transformation is more? such as algae, cyanobacteria, Arabidopsis and tobacco should be used in order to obtain clues to the metabolic and physiological consequences of a targeted genetic manipulation. Several new genome editing techniques based on the use of site-directed nucleases like TALENS (transcription activator-like effector Nuclease) or the CRISPR/Cas9 can be used for genome editing which leads to the production of plants that cannot be classified as GMPs under current legislations.

8. Conclusion

Different experimental evidences have shown that engineering of photorespiration may greatly improve plant CO_2 assimilation and growth. Several recent advances have been made in reducing photorespiratory losses in model organisms as well as in some plants of agricultural relevance. A great challenge will be the transfer of these advances to our major food crops, which are generally more recalcitrant to genetic manipulation. Nonetheless, a rational bioengineering of plants with altered photorespiration should also take into consideration that this pathway is tightly linked with several other aspects of plant metabolism, and a reduction of photorespiration may not always be beneficial, especially for plants growing under adverse environmental conditions. Nitrate assimilation depends on photorespiration, and manipulation of the photorespiratory pathway may also affect the rates of N assimilation and may favour the use of one N source over another.

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Remote Sensing-Based Monitoring of Plant Biophysical Variables and Photosynthesis Under Changing Climate

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1. Inroduction

A large part of the heat and mass fluxes within the terrestrial biosphere is governed by vegetation. The major physiological processes, such as evapotranspiration and photosynthesis, responsible within vegetation for energy and mass exchanges are driven by the canopy structure as well as the biochemistry of the foliage. For the understanding and monitoring of the typically heterogeneous and dynamic terrestrial biosphere a comprehensive and robust characterization of vegetation canopies is thus required (Sellers et al., 1997). An accurate quantitative estimation of leaf biochemical, canopy biophysical variables (such as leaf area index, leaf angle distributions and vegetation moisture content) and photosynthesis rate is of great importance for a wide range of ecological, agricultural, and meteorological applications. As they define the status of the vegetation, they are important inputs to models quantifying the exchange of energy and matter between the land surface and the atmosphere and knowledge of their spatial and temporal distribution is highly useful for regional or global-scale applications related to vegetation monitoring, weather prediction, and climate change. Accurate estimates of leaf area index (LAI), is essential in agricultural and forestry applications as LAI exhibits a major control on transpiration and uptake of CO₂ by the canopy. Retrievals of total chlorophyll content (TCab) and vegetation water content (VWC) can assist in determining vegetation physiological status and health and were found useful for the detection of

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vegetation stress, photosynthetic capacity, and productivity (e.g. Boegh *et al.*, 2002; Zarco-Tejada *et al.*, 2003). Measurement of LAI and other physiological parameters in the field is very difficult, and requires a great amount of time and efforts (Gower *et al.*, 1999). The mapping of these physiological parameters in large geographic area may be impossible when we rely on the field measurement. To solve this problem, there have been continuing efforts to develop methodologies to estimate these physiological parameters using remote sensor data. The normalized difference vegetation index (NDVI) was the most commonly used (Chen and Cihlar, 1996). Although empirical modeling is relatively easy and useful method for relating field measured biophysical parameters to remote sensor data, several factors have certain influence on empirical model (Cohen *et al.*, 2003).

The vegetated land surface is often characterized by passive optical remote sensing sensors observing the spectral properties of the surface. The spectral information content is able to provide estimates on biophysical parameters, such as Leaf Area Index (LAI) and fractional cover, as well as on parameters related to the foliage biochemistry, such as the Fraction of Absorbed Photosynthetic Active Radiation (FAPAR), up to global scale (Myneni *et al.*, 2002; Widlowski *et al.*, 2001). Recently, the active optical system LIDAR started to provide information on the vertical distribution of canopy elements within a vegetation canopy. While large footprint LIDAR capture the full vertical waveform over a canopy potentially form a spaceborne platform, airborne small footprint LIDAR can resolve the canopy structure up to a single tree. The direct LIDAR observations of vertical canopy structure can thus present an independent information source complementing the spectral information content for a comprehensive canopy characterization.

2. Biophysical Parameters Retrieval from Remote Sensing Data

Three kinds of approaches are used to retrieve biophysical parameters from Remote Sensing (RS) images : determinist modeling, semi-empirical modeling and empirical modeling. The determinist models are based on the inversion of radiative transfer models (plant canopy and underlying soil). The semi-empirical models make use in optical RS of radiative transfer models in order to build a synthetic dataset. The synthetic dataset is then used to establish statistical relationships between measured reflectances and biophysical parameters. The empirical models are based on statistical relationships built on RS vegetations indices and ground measurements of biophysical parameters.

3. Empirical Modeling

Remotely sensed data in the reflective optical domain function as a unique costeffective source for a detailed knowledge of the spatial and temporal variations of these key canopy characteristics. The shape and form of canopy reflectance spectra



depends on many factors such as vegetation structure, leaf biochemical composition, soil background, and the view and illumination geometry. For instance, LAI has a large impact on reflectance spectra especially in the near-infrared (NIR), the visible part of the spectrum is strongly affected by leaf chlorophyll, and leaf water is the prevalent factor influencing the reflectance in the mid-infrared wavelengths.

Remote sensing techniques to estimate vegetation characteristics from reflective optical measurements have either been based on the empirical-statistical approach that relates surface measurements of canopy variables to spectral vegetation indices (VI), or on the inversion of a physically based canopy reflectance model. Both approaches have their advantages and disadvantages. The potential of VIs for the determination of crop parameters have been demonstrated in numerous studies (Broge & Leblanc, 2001; Colombo et al., 2003; Gitelson et al., 2005) and the simplicity and computational efficiency of the approach makes it highly desirable for large-scale remote sensing applications. However, a fundamental problem with the VI approach for estimating biophysical variables is its lack of generality. Since canopy reflectance depends on a complex interaction of several internal and external factors that may vary significantly in time and space and from one crop type to another, no universal relationship between a single canopy variable and a spectral signature can be expected to exist. Consequently, these simple empirical relationships will be site-, time- and crop-specific, and therefore not directly applicable for large-scale operational use (Baret & Guyot, 1991; Colombo et al., 2003; Gobron et al., 1997).

Numerous vegetation indices (VIs) were developed in the framework of RS vegetation monitoring. All the existing VIs are based on the large contrast existing between vegetation reflectance observed in the red wavebands and the infrared wavebands. Such a contrast is not observed on other earth surfaces (bare soil, rocks, water bodies). As a consequence, this contrast is an indicator of vegetation presence and status. Furthermore, since vegetation is not only characterized by large reflectance values in the infra-red wavebands, but also by large transmittance values, it is pertinent to use the infra-red wavebands to get information on the vegetation under-stories. The first empirical relationships between VIs and LAI were built based on this consideration. Since then, VI's have been largely used to retrieve not only LAI, but also soil canopy coverage, leaf density, etc. A lot of different VIs were developed by many authors, in order to improve the sensibility of the indices to the parameter to retrieve, while minimizing the interferences brought by external factors such as soil background, illumination and observation conditions, atmosphere diffusion, Some well known indices are the simple ratio (SR), the NDVI, the soil adjusted vegetation index (SAVI), the weighted difference index (WDVI), etc. The main underlying issue in the use of VI's is the potential lack of generalization of the empirical relationships. A given relationship, established

in with a specific dataset, will not necessarily give good results in other situations (Iqball, 1983). The sensitivity of the VI's to LAI has been analyzed by numerous authors. For similar external conditions, the VI's to LAI relationships usually shows a logarithmic shape, with saturation of the VI for large LAI values. When the external conditions acts upon the signal, then linear relationships are more pertinently used.

4. Retrieval of Leaf Area Index

Leaf Area Index (LAI) has been an important parameter that is directly related to the photosynthesis, evapotranspiration, and the productivity of plant ecosystem (Bonan *et al.*, 1993). Measurement of LAI in the field is very difficult, and requires a great amount of time and efforts (Gower *et al.*, 1999). The mapping of LAI in large geographic area may be impossible when we rely on the field measurement. To solve this problem, there have been continuing efforts to develop methodologies to estimate LAI using remote sensor data. The normalized difference vegetation index (NDVI) was the most commonly used, however, showed the saturation phenomenon in high LAI value (Chen and Cihlar, 1996; Carlson and Reley, 1997). Although empirical modeling is relatively easy and useful method for relating field measured LAI to remote sensor data, several factors have certain influence on empirical model (Cohen *et al.*, 2003). The vegetation type, canopy structure, background, atmospheric conditions, and topographic conditions affects the empirical model.

A simulated canopy reflectance dataset for a total of six channels in visible, nearinfrared and shortwave-infrared region, corresponding to Landsat Thematic Mapper was generated using the PROSAIL (PROSPECT+SAIL) model and a range of LAI, soil backgrounds, leaf chlorophyll, leaf inclination and viewing geometry inputs. This dataset was used to develop and evaluate approaches for LAI estimation, namely, standard two-band nonlinear empirical vegetation index (VI)-LAI formulation (using Normalized Difference Vegetation Index/simple ratio (NDVI/ SR)) and a multi-band principal component inversion (PCI) approach. The analysis indicated that the multi-band PCI approach had a smaller rms error (RMSE=0.380) than the NDVI and SR approaches (RMSE=2.28, 0.88), for an independently generated test dataset.

Jianjun*et al.*, (2005) investigated the feasibility of using Landsat TM data to retrieve leaf area index (LAI). To get a LAI retrieval model based ground reflectance and vegetation index, detailed field data were collected in the study area of eastern China, dominated by bamboo, tea plant and greengage. Plant canopy reflectance of Landsat TM wavelength bands has been inversed using software of 6S. LAI is an important ecological parameter. In this paper, atmospheric corrected Landsat TM imagery was utilized to calculate different vegetation indices (VI), such as simple ratio vegetation index (SR), shortwave infrared modified simple ratio (MSR), and normalized difference vegetation index (NDVI). Data of 53 samples of LAI

were measured by LAI-2000 (LI-COR) in the study area. LAI was modeled based on different reflectances of bands and different vegetation indices from Landsat TM and LAI samples data. There are certainly correlations between LAI and the reflectance of TM3, TM4, TM5 and TM7. The best model through analyzing the results is LAI = 1.2097*MSR + 0.4741 using the method of regression analysis. The result shows that the correlation coefficient R2 is 0.5157, and average accuracy is 85.75%.

Tripathi et al., (2006) conducted a field experiment with the objectives set as (i) to relate canopy biophysical parameters and geometry to its bidirectional reflectance, (ii) to evaluate a canopy reflectance model to best represent the radiative transfer within the canopy for its inversion and (iii) to retrieve crop biophysical parameters through inversion of the model. Two varieties of the mustard crop (Brassica juncea L) were grown with two nitrogen treatments to generate a wide range of Leaf Area Index (LAI) and biomass. The reflectance data obtained at 5nm interval for a range of 400-1100nm were integrated to IRS LISS -II sensor's four band values using Newton Cotes Integration technique. Biophysical parameters were estimated synchronizing with the bi-directional reflectance measurements. The radiative transfer model PROSAIL was used for its evaluation and to retrieve biophysical parameters mainly LAI and Average Leaf Angle (ALA) through its inversion. Look Up Table (LUT) of BRDF was prepared simulating through PROSAIL model varying only LAI (0.2 interval from 1.2 to 5.4) and ALA (5° interval from 40 to 55°) parameters and inversion was done using a merit function and numerical optimization technique. The derived LAI and ALA values from inversion were well matched with observed one with RMSE 0.521 and 5.57, respectively.

5. Retrieval of Chlorophyll Content

To estimate chlorophyll content of plant, the method of indices is generally used. Indices RNIR/R700 and RNIR/R550 for chlorophyll assessment were developed as a result of signature analysis of the reflectance spectra of two deciduous species (maple and chestnut) (Gitelson and Merzlyak, 1994a, 1994b; Gitelson *et al.*, 1996) and of tobacco plant (Lichtenthaler *et al.*, 1996). They allow chlorophyll estimation in dark-green to yellow leaves within a wide range of pigment variation. It was reported also that the index R760/R695 is a sensitive indicator of plant stress (Carter, 1994). Gitelson and Merzlyak developed algorithms predicting leaf chemicals from the leaf optics validated for nine plant species in the range of chlorophyll content from 0.27 to 62.9 μ g/cm2. An error of less than 4.2 μ g/cm2 was achieved (Gitelson and Merzlyak, 1997). Increase in the amount of canopy chlorophyll, either through an increase in leaf chlorophyll content or leaf area index (LAI) will lead to a broadening of a major chlorophyll absorption well in vegetation reflectance spectra centred around 680 nm (Gitelson *et al.*, 1996).

Steele *et al.*, (2008) established a relation between chlorophyll content and the Red Edge Chlorophyll Index, described as CIred edge = [(RNIR/Rred edge) -1] based on reflectances, R, in the red edge (710 to 720 nm) and near infrared (755 to 765 nm) spectral ranges. The CIred edge, found to be an accurate measure of grape leaf Chl, is capable of predicting leaf Chl ranging from 3 to 515 mg m-2 with a root mean square error of less than 28 mg m-2. This relationship, validated using independent data sets including Edelweiss, Saint Croix, and DeChaunac grape cultivars, was very accurate in chlorophyll prediction. Such an approach has potential for not only developing simple hand-held field instrumentation but also in analyzing digital airborne or satellite imagery to assist the agricultural producer in making informed decisions regarding vineyard management.

6. Radiative Transfer Model for Canopy Reflectance

Numerous models have been developed to describe and account for the relationships between canopy bidirectional reflectance and plant biophysical parameters. Many have a foundation in the theory of radiative-transfer through turbid media, or/ and in the physical principles of geometrical-optics. It is sometimes possible to invert these so-called 'physically-based' radiative transfer models (RTF's) against a limited set of measurements of surface-leaving radiation. At different wavelengths each sampled at different solar illumination or sensor view angles, parameter values for a BRDF model can be determined and, hence used to estimate key biophysical properties of the reflecting surface especially vegetated terrestrial surfaces.

The basis of all canopy reflectance (CR) models is the physics of the interaction of solar radiation with the vegetation canopy (Goel, 1987). There are many factors that determine the canopy reflectance. These are explained as below.

7. Incoming Solar Flux

The incoming solar flux consist of two parts: The direct or specular flux, which has not been scattered by the atmosphere and the diffused flux, which has been scattered in the downward direction. The fraction of diffused incident radiation SKYL, depends on the atmospheric condition and increases with wavelength. The direction of direct flux is characterized by the solar zenith angle qs and the azimuth angle js, whereas the diffused flux is characterized by its angular distribution. Most CR models decouple the atmospheric scattering, assuming that the distribution of incident diffused flux is isotropic, and treat SKYL as a parameter. (Gerstl and Zardecki, 1985a,b).

8. Spectral Properties of the Vegetation Element

The radiation flux incident on a vegetation element e.g. leaf is subject to two



processes, scattering and absorption. Scattering consist of sub-processes, reflection from and transmission through the leaf. The reflection consist of two parts- (i) The specular reflected flux and (ii) the diffuse flux. Likewise the transmitted beam consists of refracted and diffused parts. The relative amount of diffused and specular fluxes depend upon the characteristics of the vegetation element (surface properties, cellular structure, composition etc) and those of the incident flux (wavelength, angle of incidence and polarization). Many measurements of the leaf spectral properties are available over a wide range of wavelengths (Gausman, 1985). Most of them use near normal incidence sources and integrating hemispheres that collect all the radiation emanating from top and bottom side of the leaf. These measurements give what is referred to as the hemispherical reflectance ρ , the hemispherical transmittance Γ and the absorbance (a) is given by a = 1-(ρ + Γ).

In general the radiation scattered from a leaf also depends upon the scattering direction. Only a few measurements on corn and soyabean leaves have been carried out to understand this dependence (Breece and Holmes, 1971; Norman et al, 1985). According to these measurements, scattering of radiation is non-isoptropic or non-Lambertian, the scattering is approximately Lambertian and predominantly diffused for a near normal incidence angle. Detailed estimates of specular reflectance using polarization measurements at Brewster's angle have been made by Grant *et al.*, 1983.

9. Architecture of Canopy

A vegetation element inside a canopy receives two kind of radiation such as (i) solar radiation uninterrupted by other elements and (ii) radiation intercepted and then scattered from these elements. Likewise the sensor receives several types of fluxes such as (i) flux scattered only one time from a vegetation elements (single scattering) and (ii) flux scattered several times from many vegetation elements (multiple scattering) but without reaching the soil and (iii) flux reflected from the soil and reaching to the sensor either uninterrupted by a vegetation element or if intercepted then scattered in the direction of the sensor. Therefore the CR models depend not only on the scattering and absorbing properties of the vegetation elements but also on the architecture of the canopy. Architecture of the canopy is characterized by

Spatial distribution of vegetated and non vegetated areas: The spatial distribution of the sub canopies on the ground impacts the CR because it determine which parts of the soil are lit by un interrupted solar beam and which parts are not and which parts of the radiation reflected from the soil reach to an observer (located above the canopy) uninterrupted or after being intercepted by vegetation.

Leaf Area Index (LAI): LAI is an important canopy parameter and is related to



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the canopy biomass. It is also required in models that deal with plant growth and evapotranspiration. It's manual mechanical measurement even with an electronic area meter, is quite expensive, if one wants a reasonable level of accuracy (Daughtry and Hollinger, 1984).

Leaf Angle Distribution (LAD): LAD is characterized by a distribution density function $f(\theta l, \varphi l)$, wheras θl and φl are the leaf inclination and leaf azimuth angles respectively. Thus $f(\theta l, \varphi l) d\theta l d\varphi l$ is the fraction of leaf area within the leaf inclination angles of θl and $\theta l + d\theta l$ and leaf azimuth angle of φl and $\varphi l + d\varphi l$. In most CR models, LAD is assumed to be azimuthally symmetric, a good approximation for many vegetations. It varies from one vegetation to another but for most vegetations, it can be characterized by a two parameter (μ and ν) beta distribution function given by Goel and Strebel, 1984.

Scattering from the soil: The BRDF for bare soils depends on wavelength and roughness. It is usually non Lambertian than many vegetated surfaces. The assumption of Lambertian soil is quite good for a dense canopy (LAI>3), because very little radiation reaches the ground and the effect of reflectance from soil is negligible.

10. Canopy Reflectance Model

In the early days of remote sensing, inversion of canopy models using near nadir reflectances, in several spectral bands was limited in accuracy because of fixed viewing geometry of sensors, reflectance similarity of different objects and the small number of bands. Directional canopy reflectance models of a canopy can be grouped into statistical models (Goel, 1989) and physically based models which include radiative transfer models (Goel 1989; Myneni et al, 1991; Liang and Strahlar, 1993), geometrical optical models (Otterman,1981), hybrid models (Verhoef and Bunnik, 1976, Suits, 1983) and computer simulation models (Smith et al, 1981).

Cupled atmosphere and canopy (CAC) model (Liang and Strahlar, 1993): An off nadir canopy reflectance model, the Liang and Strahlar algorithm for the coupled atmosphere and canopy (CAC) model was used to simulate multiple reflectances based on various combinations of canopy biophysical parameters. Biophysical parameters such as LAD and LAI were input to the CAC model along with reflectances of leaf and soil and aerosol optical depth. The CAC model however can only be inverted through numerical iterations and it is extremely difficult to use for retrieval of those biophysical parameters with ordinary inversion methods.

Suit Model (Suit, 1972): Developed for a homogeneous canopy. The parameters occurring in this model are the leaf reflectance and transmittance, LAI, average leaf inclination angle θl , soil reflectance ps and the fraction of diffused solar radiation

(SKYL). Suit model is an accurate representation of canopy reflectance and that the reflectance measurements are accurate. In other words Suit model is totally invertible.

GeoSAIL (Huemmrich, 2001): The hybrid radiative transfer model GeoSAIL describes the spectral canopy reflectance of a forest stand. The relatively simple GeoSAIL model was chosen due to its low computational costs and its comparable performance to e.g. the more sophisticated RTM FLIGHT (Kötz et al., 2004). The radiative transfer at foliage level is characterized by the PROSPECT model (Jacquemoud & Baret, 1990), which provides the foliage optical properties as a function of the biochemistry and is subsequently coupled with the canopy RTM. GeoSAIL describes the canopy reflectance of a complete scene including discontinuities in the canopy and shadowed scene components. GeoSAIL is a combination of a geometric model (Jasinski & Eagleson, 1990) with the SAIL model (Verhoef, 1984) that provides the reflectance and transmittance of the tree crowns. A SAIL version capable of dealing with an unlimited number of bands and multiple canopy components, such as foliage and branches, was implemented (Kötz et al., 2006). The geometric model determines the fraction of the illuminated and shadowed scene components as a function of canopy coverage, crown shape and illumination angle. All trees are assumed to be identical with no crown overlap nor does the model account for mutual shading of crowns and foliage clumping.

SAIL Model (Scattring by Arbitrarily Inclined Leaves, proposed by Verhoef and Bunnik, 1981): It is an extension of the suit model and uses fraction of leaves at discrete leaf inclination angle as parameter. This model is also totally mathematically invertible. With the use of beta distribution (for LAD) the Sail model has seven canopy parameters i.e ρ , ρs , r, SKYL, LAI, μ and ν . The key assumptions of the SAIL model are an homogeneous semi infinite medium, Lambertian reflecting leaves, leaf optical properties identical for bottom and top surfaces and leaf azimuth distributed at random.

PROSPECT (Jacquemoud and Barret, 1990) : PROSPECT is a radiative transfer model based on Allen's generalized "Plate Model" that represents the optical properties of plant leaves from 400-2500nm. Scattering is described by a spectral refractive index (n) and a parameter characterizing the leaf mesophyll structure (N). Absorption is modeled using pigment concentration Ca+b (μ g cm⁻²), Equivalent water thickness Cw (g cm⁻²) and corresponding specific dry matter content (g cm⁻²). Specific absorption coefficients (Ka+b and Kw). The parameters n, Ka+b and Kw have been fitted using experimental data corresponding to a wide range of plant types and status. Prospect has been tested successfully on independent data sets. It's inversion allows one to reconstruct with reasonably accuracy, leaf reflectance and transmittance features in the 400 - 2500nm range by adjusting three input variables N, Ca+b and. Cw.



PROSAIL (PROSPECT +SAIL) Model (Jacquemoud, 1993)The canopy spectral reflectance $R(\lambda)$, as calculated by this Model depends on the following parameters:

- i. Biophysical parameters: The quantities Ca+b, Cw, N, LAI and θl .
- ii. Soil spectral reflectance $\rho s(\lambda)$
- iii. External parameters: Zenith ($\theta 0 = 00$) and azimuth ($\varphi 0 = 00$) viewing angles, Zenith illumination angle ($\theta s = 400$) and fraction of diffused incident radiation (SKYL).

In comparison with previous work, the improvement of this model is that leaf and canopy optical properties are now described in terms of biological characteristics (chlorophyll and water content). Thus in principal, physiological processes of the plant canopy such as photosynthesis or water stress status can be directly related to remote sensing data.

Leaf optical properties are the common input parameters, which are common to all canopy reflectance models. One dimensional radiative transfer models proved to meet these requirements (Myneni and Ross, 1991). For this one of the most popular model is SAIL model (Verhoef, 1984, 1985). Other models are IAPI model by Iaquinta and Pinty (1994). The Kuusk model based on a markov chain approach (Kuusk, 1995b) to describe the architecture and the NADI semidiscrete model published by Gobron et al (1997). Three different leaf angle distribution (LAD) were used- continuous ellipsoidal (SAIL) or elliptical LAD (Kuusk, 1995), and six discrete Bunnik functions (IAPI and NADI) that are supposed to cover a large range of leaf inclinations. Finally these models have been coupled to PROSPECT model and renamed PROSAIL, PROSIAPI, PROKUUSK and PRONADI.

11. Inversion of Canopy Reflection Model

Inversion of canopy models using off nadir data based on studies of BRDF has been the primary concern for quantitative extraction of biophysical parameters of canopy architecture (Goel, 1988; Liang *et al.*, 1993). All physical canopy reflectance models are not invertible. An ideal model for inversion purposes should comply both criteria of accuracy (in the sense it should represent correctly the radiative field within the canopy) and speed. For inversion different strategies have been proposed each owing specific advantages and disadvantages (Kimes et al, 2000) and these are

- i. Numerical optimization methods (Bicheron and Loroy, 1999).
- ii. Look Up Table based approaches (Combal et al., 2002; Weiss et al., 2000)
- iii. Artificial Neural Networks (Atgberger et al, 2003a ;Weiss et al., 2000).
- iv. Principal Component Inversion technique (Satapathy and Dadwal, 2005)

A good model is a compromise between a few parameters and good fit for traditional inversion purposes. Therefore, a model inversion that lasted several minutes to several hours was not satisfying, regardless of whatever accurate result is produced.

For that very reason, PRONADI was discarded for our inversions on field data. An approach using neural networks or look up tables for instance might produce different conclusions. If the running time prevails over accuracy, then PROKUSSK< PROSAIL < PROSIAPI< PRONADI. If the accuracy on Ca+b is considered as the main factor then PROSAIL < PROSIAPI< PROSIAPI< PROSIAPI< It is the accuracy on LAI, then PROKUSSK< PROSAIL < PROSAIL < PROSIAPI. The differences however are less noticeable than those for running times.

Though many work have been done abroad. In India hardly any work is done on inversion except studying the effect of sensor geometry on bidirectional reflectance and its relation with biophysical parameter like LAI and dry biomass (Chakraborty, 2002), validation of PROSAIL model (Barman, 2005, Dadhwal, 2005) and LAI and leaf angle distribution retrieval of mustard through inversion of PROSAIL model (Tripathi *et al.*, 2011; Tripathi *et al.*, 2013).

12. Remote Sensing for Estimation of Photosynthesis in Rice

Net photosynthesis rate (PN) has an important role incontrolling the ecosystem primary production and it isimportant to understand the relationships between PNand other physiological and environmental variablesto improve and develop models for predicting plantgrowth and productivityThere is a need for nondestructive and rapid method for assessment of leaf photosynthetic characteristics to support photosynthesismodelling and growth monitoring in crop plants. Tian et al. (2005) determined the quantitative relationships between leafphotosynthetic characteristics and canopy spectral reflectance under different water supply and nitrogen applicationrates. The responses of reflectance at red radiation (wavelength 680 nm) to different water contents and nitrogen rateswere parallel to those of leaf net photosynthetic rate (PN). The relationships of reflectance at 680 nm and ratio index ofR(810,680) (near infrared/red, NIR/R) to PN of different leaf positions and leaf layers in rice indicated that the top twofull leaves were the best leaf positions for quantitative monitoring of leaf PN with remote sensing technique, and the ratioindex R(810,680) was the best ratio index for evaluating leaf photosynthetic characteristics in rice. Testing of the models with independent data sets indicated that R(810,680) could well estimate PN of top two leaves and canopy leaf photosynthetic potential in rice, with the root mean square error of 0.25, 0.16, and 4.38, respectively.

In another study, Mamta et al (2012) used ground-based hyperspectral measurements for estimatingnet photosynthesis during the growth cycle of rice.Groundbased hyperspectral reflectance and micrometeorologicaldata (PN, chlorophyll concentration

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index (CCI), photosynthetically active radiation (PAR))were collected from a rice field on three dates in thekharif season. Greenchlorophyll index (CIg) showed a positive and statistically significant relationship with CCI (R2 = 0.64). Useof single index showed poor fit with PN. The modelbased on a combination of two variables (vegetationindex (VI) × PAR) however proved better in explaining the variance of PN.

13. Conclusion

The biggest challenge for validation of moderate and coarse resolution satellite products is the extrapolation of source field data from sampling points to a sufficiently extensive area. One way of doing this is to employ both field measurements and high resolution satellite data and field measured biophysical parameters at sampling points is the key element in the implementation of this strategy. Additional studies covering more sites and vegetation types to be conducted and necessary correction may be recommended for algorithm refinement of estimating biophysical parameters and photosynthetic rate before its operational use.

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Farming Interventions for Addressing the Climate Change Induced Sea Level Rise Concerns of Coastal India

B. Gangaiah

1. Introduction

Coastal area means 'area of land within a distance of two km from the High Tide Line of seas, rivers, creeks and backwaters'. It is thus the interface or transition regions of land and sea with specific features like inland lakes, estuaries, lagoons, mangroves, backwaters, salt marshes, rocky coasts, sandy stretches and coral reefs etc. They have diverse functions and some of them (ports, sports fishing and tourism) are exclusive to them. Unlike watersheds, coastal areas have no exactly fixed boundaries. India is bestowed with 8118 km of coastal area (over 74.8% encircling the mainland of country in 9 states and 2 union territories (UTs) in 63 districts starting from Gujarat on the West ending in West Bengal on the East and rest 25.2% in Andaman & Nicobar Islands lying in Bay of Bengal and Lakshadweep Islands in Arabian Sea). The islands lying in the midst of ocean was encircled by coast all around with unique features of live coral reefs, whereas the mainland states have coast on one side only. The Exclusive Economic Zone (EEZ) of coasts i.e. 2.02 m km2 (FAO, 2000) extending from appropriate base line from land towards the ocean up to 200 nautical miles (of which up to 12 nautical miles are territorial waters with 0.506 m km² continental shelf exploited by many small fisherman) makes them more important for fish, marine organisms including sea grass, salt, petroleum and seabed minerals. On landward side of the coast, dense human habitations have developed to house 360 m people (2001 census) and farmers of these areas practice Coastal artisanal fishing mixed farming system type agriculture. Artisanal fishing entails primitive tools based fish capture; however, at many places

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in mainland states mechanised commercial gears have come up for fishing in recent past necessitating earmarking of fishing areas for small fisherman. Crops, livestock and aquaculture and commercial mariculture (shrimp or prawn) activities are the components on which mixed farming systems was built.

2. Agro-Ecosystem of Coastal India

Coastal areas are spread in 3 agro-ecological zones (AEZs) of India i.e. Eastern Coastal Plains (AEZ 18); Western Ghats and Coastal Plains (AEZ 19) and Islands Regions (AEZ 20) on 20.4 m ha geographical area (6.5% of country) of which 58.2% i.e. 11.87 m ha was gross cultivated area (Sehgal et al., 1992). The coastal states / union territories of India are shown in Figure 1 and are detailed in Table 1. As per 2011 census; 4 of the 8 metro cities (population >4 million) i.e. Mumbai, Chennai, Kolkata and Surat with sea ports and 5 of the 38 million plus population cities i.e. Howrah, Visakhapatnam, Navi Mumbai with sea ports and Thane, Vijayawada without sea ports of India are located in coastal regions indicating not only the population pressure but also their prominence as economic nodes stimulating growth and development of surrounding regions. High rainfall of AEZ 19 and 20 (200-320 cm) provides over 210 days of length of growing period (LGP) with multi-storey plantation cropping (coconut / areca nut with spices, tubers, ginger and turmeric etc.) at high latitudes and annual cropping of rice, vegetables etc. in the plain and coastal periphery area in lower altitudes. The AEZ 18 with 120-190 cm rainfall has a LGP of 60 - 210 days where annual crops (rice, fruits, and vegetables) has predominance to plantation crops. The major dams constructed across the rivers in East coast make it a food grain production zone. Fertilizer use, a function of mode and intensity of cropping was higher in AEZ 18(144.7) than AEZ 19 (80.3) and AEZ 20 (14.3) as compared to the countries average (89.8) in 2004-05 (FAO, 2005).



Fig .1 Coastal ecosystem of India

State / Union Territory	Name of district
West coast STATES/ UNITON TERRITORIES (UTs)	
Gujarat (9)	Porbandar, Navsari, Bharuch, Amreli, Valsad, Kachchh, Anand, Bhavnagar, Surat
Daman and Diu (2)	Daman and Diu
Maharashtra (5)	Sindhudurg, Ratnagiri, Raigarh, Greater Bombay and Thana
Karnataka (3)	Udupi, Uttar Kannada and Dakshin Kannada
Goa (2)	South Goa and North Goa
East Coast STATES / UTs	
Kerala (10)	Kasaragod, Kottayam, Alappuzha, Kannur, Kollam, Kozhikoda, Ernakulam, Thrissur, Thiruvananthapuram and Malappuram
Tamil Nadu (11)	Thiruvarur, Ramanathapuram, Pudukottal, Nagapattinam, Thoothukudi, Kanyakumari, Cuddalore, Thruneivell, Villupuram. Kancheepuram and Chennai
Pondycherry (3)	Mahe, Karaikal, Yanam
Andhra Pradesh (9)	Srikakulam, Vizhianagaram, Vishakapathnam, East Godavari, West Godavari, Krishna, Guntur, Prakasam and Nellore
Odhisa (5)	Jagatsinghpur, Bhadrak, Kendrapara, Baleshwar and Ganjam
West Bengal (4)	Howra, South 24 Parganas, North 24 Parganas and East Midnapore
Island UTs	
Andaman & Nicobar Islands (3)	North & Middle Andaman, South Andaman, Car Nicobar
Lakshadweep (1)	Kavaratti

Table 1 Coastal states and districts of India

Livestock (diary, sheep, goat: 53.3 m), poultry (130 m) and piggery (roughly 3, 42, 378 in all districts except in Lakshadweep), the liquid assets of farmers by recycling crop by-products, feeding on grasslands and cultivated fodders produce nutritious food (milk, meat, eggs) for human beings besides promoting crop culture through providing draught power, manures and organic pesticides like panchagavya etc. Owing to these multifunctions of animals, they are integrated with crops culture from ancient times. Not only land owners, but landless people rear animals and reaps the rich benefits. Few of the land less people practice commercial dairy / poultry rearing activities based on purchased inputs while rest of landless farmers depend on community lands and weeds and purchased straw for livestock rearing.

The whole marine capture fish (3.83 m t in 2017-18) emanates from coastal regions with West coast contributing over 63% of it (Sathianandan, 2016). Aquaculture (in estuaries, deltas, farm ponds etc.) was also prominent in mainland coastal states of India in addition to marine fishing. Brackish water aquaculture was practiced on 13% of the estimated potential area of 1.2 m ha. Prawn / shrimp culture was



also popular in East coast of India that by way of 0.435 m t shrimp export during 2016-17 has earned 3.726 billion US \$ to the country. There were 6.7 m full-time, part-time and occasional fishermen in coastal areas of India (Vivekanandan, 2002).

Tourism including agro-tourism was exploited in Goa and Maharashtra. Among islands; Andaman & Nicobar Islands stands better than Lakshadweep in tapping tourism sector including sports fishing tourism. Sports tourism was also emphasized along Andhra Pradesh coasts especially in Visakhapatnam in recent times. However, this unique ecosystem on which many livelihoods are dependent (especially marine fisherman, farmers) was threatened by various vulnerabilities.

3. Vulnerability of Coastal Areas

Coastal regions are vulnerable to various contingencies from historical times. Invasion of foreign rulers' was such menace; that was more so in times when maritime capabilities decide the countries power and its command on world. The reaching of Portuguese navigator Vasco da Gama and his team to Kappakadavu, near Calicut, Kerala, India on 17th May, 1498 has succeeded in the establishment of direct sea route from Europe. It was followed by establishment of trading posts by the The Dutch Republic, England, France, and Denmark-Norway. Subsequently India came under East India Company rule and finally under direct British rule after 1857, Sepoy mutiny in 1858 that continued till independence in 1947. The natural vulnerabilities of coastal region include cyclones and associated floods, earthquakes, volcanic eruptions and climate change. Some of the most cyclones of 21st Century (Nilam, Phailin, Hudhud, Ockhi, Titli of October, 2011, October, 2013, October, 2014, November-December, 2017, and October, 2018) struck the coast devastated the coastal areas. Heavy rainfall could also bring in immense vulnerability to coastal regions as evident from the Kerala floods, when 30 cm rainfall was received in a day on 15th August, 2018. East coast being close to the Pacific Ring of Fire (an area of frequent earthquakes) experiences more earthquakes. Oceanic earthquakes and volcanic eruptions often are associated with Tsunami. The 26th December, 2004 - Indian Ocean tsunami following Sumatra, Indonesia massive earthquake (9.3 magnitude on Ritcher scale) was the devastating memory of coastal Indian states especially in Andaman & Nicobar Islands (ANI). The ANI also has the only active volcano of the country in Barren Islands that became active again on 25th September, 2018 by spewing lava and ash. Accelerated anthropogenic activities of post-industrial periods have increased the sea surface temperatures by 10C and as per the IPCC Special Report on Global Warming released on 8th October, 2018 calls for urgent corrective measures to limit the temperature rise to 1.50 C by 2030 (Jonathan Watts, 2018). The increased temperatures are resulting in melting the polar ice. Enhanced volume of such melted water from ice in the past century has brought in 0.2 m sea level rise globally (Church et al., 2013) and this rise will continue in

coming periods too. Raised sea levels by way of inundating low elevation areas of coasts bringing in innumerable difficulties to the inhabitants livelihoods and thus requires concerted global efforts to restrict future sea level rises through mitigative and adaptive measures. The submerged city of Dwaraka, in Gulf of Cambay, Gujarat stands as reminisce of such sea level rise in the past. Thus climate change is the most serious threat to the coastal regions all over the world including India. The massive earthquake of 26th December, 2004 followed by tsunami of Sumatra, Indonesia has created sea level rise situation abruptly in Andaman & Nicobar Islands (ANI) in contrast to the gradual sea level rise associated with climate change with effects like biota redistribution. If we set aside the causative factors of above two factors induced sea level rises, the coping mechanisms would be identical. Thus the coping strategies evolved for earthquake subduction landmass of ANI are also applicable to climate change-sea level rise.

4. Land Subduction from 2004 Earthquake in Andaman and Nicobar Islands

Convergence movement of the Indian and Burmese earth's plate (the former moving beneath the later plate) has caused 2004 massive earthquake of Indonesia that resulted in the tilting of whole land mass of ANI (part of Indian plate) in anticlockwise rotation by about 2-3 m. Therefore, the Southern part of the islands landmass has undergone subsidence up to 2 m while the Northern Andaman parts have registered 1 m uplift of landmass from sea bed. Land subsidence was seen in Nicobar and South Andaman districts on Eastern coast. Great Nicobar Islands, the Southernmost Island of India (Nicobar district) has recorded maximum subsidence (3 m) as evident from the submerged light house into water. Similarly, Kamorta and Trinket islands, Nicobar district too have recorded land subsidence and the later islands got bifurcated with sea water entry into the low lying area. In South Andaman districts; Sipighat, Chouldari, Krikabad, Badmaspahar, Portmout, Muslim basti, Ograbraj, Mithakhari, Namunaghar, and Dundus point have experienced the land subduction. Coral beds and trees submergence up to 1.5 m was noticed at Rangat, N & M Andaman district. This subduction of landmass into ocean has resulted in inundation on the coastal stretches as much as 2 km in some cases.

The 4206 ha of farmlands were in those inundated areas. Additional, 3863 ha farmlands was also got under influence of ocean waters that however, could be reclaimed. In total, over 6300 farmers and their livelihoods were devastated (Table 2). Many areas also got submerged in ocean water duirng high tides that get receded at low tide. These submerged areas have impacted the drainage of lowlands that became water ponds. The sea water ingress underground also affected utility of many farms lands.



Name of the Islands	Submerged area (ha)			Reclaimable area (ha)				No. of
	Paddy	Plantation crops	Total (A)	Paddy	Plantation crops	Total (B)	Total (A + B)	affected farmers
S.Andaman	1148	131	1279	319	69	388	1667	1448
L. Andaman	-	-	-	43	74	117	117	48
Rangat	11	-	11	73	-	73	84	181
Mayabunder	5	7	12	39	7	46	58	19
Diglipur	-	-	-	27	-	27	27	47
Car Nicobar		213	213		756	756	969	2378
Kamorta		527	527		110	110	637	341
Nancowrie		244	244		13	13	257	251
Trinket		289	289		40	40	329	140
Teressa		343	343		401	401	744	268
Chowra		58	58		173	173	231	340
Bambooka		20	20		10	10	30	15
Katchal		331	331		1297	1297	1628	317

Table 2 Distribution of 2004 Earthquake and tsunami impacted farm acreage and farmers in

 Andaman & Nicobar Islands.

(Adapted and modified from Digal, 2006)

669

2831

211

1375

Campbell bay

Total

5. How Earthquake Impacts of ANI are Related to Climate Change

880

4206

301

803

110

3060

411

3863

1291

8069

525

6324

Permanently submerged land mass of ANI into the Indian Ocean (Situation I) on its landward side has additional ocean submerged areas during high tide. These high tide impacted areas besides becoming saline also made nearby lands and water bodies saline through subsurface water ingress (Situation II) that severely impaired their agricultural utility. The above two situations caused impeded drainage of coastal lowlands and turned them into ponds (Situation III) filled with water that was neither saline nor fresh type and making them out of cultivation. Various coping mechanisms were evolved to restore agriculture in above earthquake induced situations of Islands. Nicobar Islands of ANI and Lakshadweep Islands with near to sea level altitudes are anticipated to bear the wrath of sea level rise in future in India. The studies made by various organizations after 2004 earthquake cum tsunami in ANI especially ICAR-CIARI, Port Blair through institute, National Agricultural Innovation Project (NAIP), 'Strategies for sustainable management of degraded costal land and water for enhancing livelihood security of farming communities' (under component 3) and National Innovations in Climate Resilient AgricultureCompetitive Project (NICRA) - Competitive Grants Project (CGP) on restoration of agriculture in tsunami affected lands of Andaman and Nicobar islands have given conclusive leads to re-practice agriculture in the devastated lands.

6. Coping Strategies / Technologies Evolved for three Situations mentioned above

Adoption was the immediate attempt made by all the stakeholders for the contingency created as mitigation of earthquakes and tsunamis are beyond human control. Human dwellings inundated under inundated ocean water during earthquake cum tsunami were relocated to the elevated regions of the same Islands or to other Islands at huge cost. Trinket island people were got vacated and resettled in Kamorta Island. This will holds true for climate change adoption mechanism also. All the current and future development activities need to be located at safer elevations that should be well above the anticipated sea level rise. The major interventions evolved for restoring agriculture in tsunami impacted lands till date in Islands and elsewhere in the country are described below.

6.1. Shore Embankments / Barriers for Coastal Protection and Productive Land Use

To protect the shallow water and high tide submerged farm lands and habitations; shore embankments were made along the coast by all tsunami affected states of India. In this direction, utility of bio-shields (both natural and planted vegetation) was vastly explored. Mangroves are the natural bio-shields of coasts, whose restoration would protect the coastal areas. Submergence of the well established mangroves with subduction of landmass of ANI into ocean has increased the depth of waters and salinity leading to mortality of mangrove vegetation. At the same time mangroves are developing in the newly submerged areas having appropriate depth and salinity. Development of bio-shields was emphasized looking at their protection functions i.e. few or no human casualties and infrastructure damages in mangrove and other vegetation containing coasts in 18 tsunami-affected hamlets along 25 km coastline of Tamil Nadu (Kathiresan and Rajendran, 2005). Danielsen et al. (2005) from experiences of Cuddalore district, Tamil Nadu reported Casuarina as the best candidate tree for bio-shield plantation all along the coasts. Accordingly along Andhra Pradesh (AP) coast, Casuarina monocultures were planted that have now fully grown up. In ANI too, on the earthern embankments constructed with sluice gate by administration at most of places to contain the sea water ingress into farm lands, were strengthened by planting Casuarina on both sides of embankment (Figure 2). Many non-government organizations (NGOs) that started functioning in tsunami affected states like Tamil Nadu (TN) and in Kerala (by forest department) have given emphasis on mangrove species (Avicennia marina or Rhizophora spp.)

monocultures. The native vegetation of coasts like Ipomea biloba a creeper on all coasts and Pandanus also called as screw pine; a monocot shrub seen in ANI coasts with firm sand holding, dune formation and tidal protection powers needs to be explored as bio shelters. However, in Kerala (Northern parts) locally available species planting in mixed cultures was stressed upon. In ANI, gabion structures (Figure 3) were developed along the coast in tsunami regions for erosion protection and containing wrath of tides. In Kerala state, major emphasis was given to sea wall construction after tsunami (550 km of total 600 km coastline has walls).



Fig. 2 Casuarina plantation on strengthening earthen embankment with sluice gate at Ograbraj, South Andaman, ANI (Source: B. Gangaiah)



Fig. 3 Gabion structure erected along the coast after tsunami for protection at Chouldari, ANI (Source: B. Gangaiah)

6.2. Utilization of Permanently or High Tides Ocean Submerged Areas

Bringing permanently or high tide submerged lands to utilization poses a great challenge. Successful solutions were evolved through innovative approaches to tap the emerging opportunities for such areas and most important of them have been detailed below.

Sea weed culture

Sea weeds (Marine macro algae) are the non-flowering plants of intertidal / sub-tidal regions and constitute one of the commercially important renewable marine living resources with multiple uses as source of food, energy, chemicals and medicines. Sea weeds provide raw material for health food, medicines, pharmaceuticals, textiles, fertilizers and animal feed industries. From the phycolloids (agar, carrageenan, align); food, livestock feed additives, soil amendments and fertilizers (especially potassium). Owing to the minerals, vitamins, trace elements and bioactive substances richness, seaweeds are often referred to as the medical food of the 21st century. Current sea weeds market (US \$ 10.6 billion in 2016) with a compounded annual growth rate of 10.8% was anticipated to touch US \$ 26.1 billion by 2025. China tops in seaweed production, followed by North Korea, South Korea, Japan,



Philippines, Chile, Norway, Indonesia, USA, India together these ten countries contribute about 95% of the world's commercial seaweed volume and 90% seaweed production comes from their culturing. In ANI, 27 genera of sea weeds were reported (Anuraj et al., 2016). South India, Islands and Gujarat coast are rich in sea weed distribution and thus scope lies for their exploitation. Commercial culture technologies were developed by CSIR- Central Salt and Marine Chemical Research Institute (CSMCRI), Bhavanagar, Gujarat for Kapppaphycus and Gracilaria that figure in the top 5 cultivated seaweed species of the world. Similarly for agar yielding Gracilaria edulis and Gelidiella acerosa, CMSCRI Marine Algal Research Station (MARS), Mandapam, Tamil Nadu (TN) and for Hypneamus ciformis and Acanthophora spicifera, ICAR- CMFRI, Cochin have culture technology standardised. K. alverezii farming that became common along the Coromandel Coast (South East India) in Gulf of Mannar could be extended to other sea weeds and areas in Tamil Nadu, Orissa, Okha Veraval and Konkan coasts. Studies have indicated that tsunami inundated areas of Bimblitan and Kadakachang of ANI (Anuraj et al., 2017) as suitable places for seaweed culture. Globally seaweed are grown by two methods i.e. Single Rope Floating Raft and Fixed Bottom Long Line Integrated Multi Trophic Aquaculture methods. In India, Single Rope Floating Raft (Coir Rope & Nylon Rope) method was most popular and the last method was least popular. Submerged and floating sea weeds culture systems of coastal areas of Kerala, Tamil Nadu, Minicoy (Lakshadweep) etc are depicted in Figure 4a & 4b. Integrated sea weed cum commercial finfish and shellfish culture (Figure 5) with high system productivity (25, 50 and 30 tonnes of fish) and revenues was evolved. The adverse environmental of industrial mariculture on water quality was taken by sea weeds though their purifying acts. Most important sea weeds are shown in Figure.6.



Fig. 4a. Raft method of sea weed culture at Erwadi, Tamil Nadu: (Source: The Hindu)

Fig. 4b. Floating Raft method of sea weed culture at Orissa: (Source: CES, 2012)

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Fig. 5 Integrated fish and sea weed farming at Munikadu (Source: The Hindu, 3rd November, 2014)



Acanthophora spicifera

Fig. 6 Important sea weeds

Amphiroa anceps

Gracilaria sp.

Enormous scope of sea weed products/ extracts in agriculture and industry adds to its commercial success of such ventures. The biostimulant extracted by CSMCRI, Gujarat from Kappaphycus alvarezii and Gracilaria edulis owing to its richness in growth promoting substances and nutrients(Layek *et al.*, 2015) evaluated in multi-location and multi-institute field tests has indicated a crop yield enhancements of 10-37% (Table 3).

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Constituent	Amount in mgL-4	Amount in mgL-4			
	Kappaphyeus alvarezii sap	Gracilaria edulis sap			
Indole 3-acetic acid (IAA)	27	8.7			
Zeatin	20	3.1			
Gibberellin (GA3)	24	-			
Choline	57	36			
Glycine betaine	79	63			
N+	198	1952			
К+	33654	682			
(a 2+	321	352			
Mg 2+	1112	311			
Zn 2+	4.7	0.63			
Mn 2+	2.1	33			
Fe 2+	86	13			
(r 3+	32	0.20			
Cu 2+	0.65	0.04			
Ni 3+	3.5	0.21			
P3+	17				

Table 3 Composition of two vastly exploited sea weeds

6.3. Fish farming

Shrimp or brackish water aquaculture was found more suitable activity to ocean subducted regions of earthquake and also for climate change induced sea level rise regions too. The brackish water shrimp culture suitable area (608 ha) of ANI was increased by 1,206 ha after 2004 earthquake cum tsunami. The water level maintenance for brackish water culture (minimum water depth of 1 m) was not an issue in land subduction zones; however, for maintaining such water level in high tide influenced regions, construction of bund or dyke with self operated sluice gate system facilitating entry and exit of ocean water was essential. Candidate species include seabass, shrimp, mud crab, mullets for permanently submerged areas while shrimps are suitable for high tide submerged areas. No severe losses from white spot syndrome virus (WSSV) as that of mainland in shrimps (Litopenaeus vannamei) was an added advantage for ANI. With proper management, a shrimp production of 1.5-2.0 tonnes /ha can be realized in Andaman's.

Mud crab fattening

High tide inundated areas were ideal for fattening of lobster and crab that fetches a premium price in the market. In crab fattening systems, juvenile lobsters and crabs like Scylla serrata, S. tranquebarica that are freshly moulted (water crabs) weighing



550 g are collected from the wild and were grown to marketable size by stocking @ 1/m2 in brackish water ponds and individual cages / tanks for a month period. Trash fish and other discards like poultry meat etc. are fed to crab @ 5-10% body weight, 2-3 times a day. Selective harvesting for need based marketing was followed. Low operating costs and fast turn over periods makes this venture highly rewarding.



Fig. 7 Crab fattened in ponds (Source: Dr. Nagesh Ram, KVK, Sippighat)

Studies on crab fattening in high tide impacted areas in post-earthquake cum tsunami periods was made by KVK, Sippighat, South Andaman during July, 2017 through digging a pond. A sluice gate was created to take sea water during high tide that retreats during low tide. Sluice gate was erected at a higher elevation than the bottom of pond so as to maintain the minimal water depth continually. The daily water exchange between sea and pond resulted in maintenance of sea water salinity levels (Figure 7). The findings of the study have revealed the rich economic dividends (Annual Report, 2017-18) in 17 days (Table 4). Over a year, 20 cycles of crab fattening can be done. However, inadequate seed material of crabs for fattening was hindering its wider and full exploitation. Breeding and seed production technology developed for crab (S. tranquebarica)at ICAR-CMFRI, Cochi with 20 per cent survival of larvae from eggs gives a boon for the crab fattening activities.

Parameter	Value
Number of crabs released (crabs harvested)	50 (46)
Cost of released crab (Rs/kg)	450
Culture period (days)	17
Harvest weight of crab (kg)	70
Feed cost (ray fish feeding @3 kg /day costing Rs. 30/kg)	1530
Labour cost (Rs)	12,000
Total cost (Rs/ 17 days) of production	13,530
Gross revenue from crab sale (@ Rs. 1500 /kg)	1,05,000
Feed conversion ratio	4.0
BCR	7.76

Table 4 Economics of crab fattening in high tide impacted in South Andaman

6.4. Integrated Mangrove Fishery Farming System (IMFFS) Development

Mangroves are the salt tolerant trees and shrubs that exist at the interface between land and sea in the tropics and sub-tropics known for high salinity, extreme tides, strong winds, high temperatures and muddy anaerobic soils. Indian mangrove forests spread on 4482 km2 area (57, 23 and 20% on East, West coast and ANI) are known for their protection, of coasts, water enrichment, fish breeding etc. functions. The mangrove vegetation roots i.e. stilt roots of Rhizophora species (prevent the intrusion of salt water and arrest wind speed during the cyclone and flood periods) and exposed surface of the breathing roots of Avicennia, Sonneratia and Lumnitzera with numerous lenticels (for passive oxygen diffusion) aid in prevention of soil erosion. The tsunami wave's energy got absorbed / diverted by mangroves that prevented the human casualties on landward side. These invisible functions of mangroves came to the notice of all only during 2004 tsunami. For example, the Coringa mangroves of East Godavari district in the Godavari estuary, near Kakinada on 237 km2 has suffered the least house collapses, human casualties etc. Studies have revealed that mangroves are more effective to concrete barriers in reducing erosion, trapping sediments and dissipating the energy of breaking wave. In ANI, mangroves ecosystem got disturbed / destroyed with subduction of land into ocean. The destruction of submerged mangroves was ascribed to increased water depths, altered salinity and sediment flow (Figure 8). The loss of fish breeding grounds and its catch to fisherman from such altered ecosystems of mangroves has resulted in their neglect.

Mangrove survival / protection could be ensured by stakeholders if fish catch was ensured and to bring this synchrony, Integrated Mangrove Fishery Farming System (IMFFS) was evolved. In IMFFS, ponds are constructed in degraded mangrove regions with planting of mangrove species on bunds and brackish water fish rearing


Fig. 8 Dying manngroves due to land subduction enhanced water depths in ANI (Source: Dam Roy, ICAR, CIARI)

in pond (Figure 9). Established mangrove vegetal cover will provide the ecosystem services while the frequently harvested fish (like sea bass) generates sustainable income and employment to local residents. M. S. Swaminathan Research Foundation (MSSRF) with support from The International Union for Conservation of Nature (IUCN) has tested IMFFS utility at Rameshwaram, Tamil Nadu and Sorlagondi, Nalli and Basavanipalem Villages of Krishna Mangrove Wetlands, Andhra Pradesh. Under NICRA-CGP project implemented in ANI, the utility of IMFFS was assessed in newly developing mangrove areas under influence of high tide at Ograbraj, South Andaman (Figure 10). A pond was dug with establishment of coconut on bunds and fish in pond. At Rampur, N & M Andaman, the tsunami devastated land adjacent to the sea with mangrove species was intervened through pond (29 m x 15 m x 3 m; L x B x D) development (Figure 11a). In pond, fish (Magur) culture was taken up and on the bed formed by excavated soil levelled as an emarkement on all sides of the pond; coconut +banana +vegetables were established (Figure 11b). Poultry shed was added as component. Economics are to be worked out while all the components were successfully established. The restrictive land use regulations are hindering the effective and economical use of submerged coastal areas in ANI.

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Fig. 9 Sea bass harvest from IMFFS unit (Source: IUCN and MSSRF)

Fig. 10 IMFFS site at Ograbraj, South Andaman (Source: B. Gangaiah, ICAR, CIARI)



Fig. 11a Pond made



Fig. 11b Water filled pond with banana,other crops

Fig.10 Pond based intervention at tsunami affected mangrove site in Rampur, N & M Andaman under NICRA-CGP project (Source: B.Gangaiah, ICAR-CIARI)

6.5. Salinity Tackling Interventions for Tsunami Impacted Lands

Salinization of soil, water and biotic resources was the most visible disturbance to farming in tsunami impacted lands as evident from the studies of Raja *et al.* (2009) in ANI and also from Nagapatnam district, Tamil Nadu (Annual Report, 2015). High rainfall (300 cm) of ANI on coarse textured slightly acidic soils was partially successful in diluting the salts from high tide impacted (both above and below ground saline water ingressing) sites after tsunami (Table 5) making them fit for reviving farming within two years. However, intermediate soil and water quality i.e. neither normal nor saline (due to continuous mixing of rain and ocean water) was hindering the marine / fresh water aquaculture activities. For such changes to happen in soil and water resources of main land states, use of soil amendments, salt affected soils / water resources utilization solutions evolved must be adopted. Crop selection was the first step towards successful farming in these areas. The important crops and their abilities to cope with salinity and water (excess) stress are detailed below.

Parameter	February and March 2005		February and March 2007	
	Surface soil (0-15 cm)	Subsurface soil (15-30 cm)	Surface soil (O- 15 cm)	Subsurface soil (15-30 cm)
pН	6.3±0.69	6.2 ± 0.59	5.9±0.62	5.7±0.80
ECe (dSm-1)	21.7±9.87	19.6 ± 7.05	6.2 ± 1.57	5.6 ± 1.33
Na+(meql-1)	191 ± 81.5	174±59.9	53 ± 15.6	47 ± 12.9
Ca2++Mg2+(meql-1)	32 ± 9.5	<i>30</i> ± <i>8.0</i>	15 ± 3.0	15 ± 2.8
SAR	46 ± 14.9	44 ± 10.0	19±4.2	17±3.5
HCO-3(meql-1)	2.4 ± 0.97	2.4±0.71	0.3 ± 0.19	0.4 ± 0.26
Cl- (meql-1)	147 ± 57.6	138±42.9	42 ± 10.4	38±8.8
SO42- (meql-1)	75 ± 34.8	67±26.3	25±7.3	21 ± 6.4

 Table 5 Soil salinity and other parameter changes in tsunami affected areas into which sea

 water ingresses daily during high tide with time

6.6. Paddy Crop Tuning to Tsunami Lands and Soils

Paddy was the inevitable choice for cultivation in saline and excess water stressed regions. In this direction to promote paddy cultivation in tsunami soils in ANI, two salt tolerant varieties (CARI Dhan 4 for saline soils and CARI Dhan 5 acidic saline soils) were evolved through induction of somaclonal variation in Pokkali land race. In addition, 2 pure lines (CARI Dhan 8 and CARI Dhan 9) from local 'C-14-8' variety that is known for its long duration and photosensitivity suiting for low lying areas were also developed (Gautam *et al.*, 2014). These varieties preferred by farmers from participatory studies were brought under truthfully labelled (TFL) seed production. During 2012-17, 17.67 t of TFL seed of 5 rice varieties (Table 6) was produced and distributed to farmers through agriculture department (Singh *et al.*, 2014). Water logging tolerant, deep water rice varieties of ICAR-NRRI, Cuttack (Jaldhara and Jalmagna etc.) and salt tolerant varieties evolved by ICAR and SAUs (ICAR-CCSRI Canning, West Bengal, ANGRAU, Pulla, Andhra Pradesh, TNAU, Tirchurapall, KAU, Vellanikkara etc.) could be used for paddy cultivation in mainland states.

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Variety	Production (t)	Period
CARI Dhan 4	4.85	2012-17
CARI Dhan5	5.61	2011-17
CSR 36	6.74	2011-17
CARI Dhan 8*	0.22	2015-17
CARI Dhan 9*	0.25	2016-17
Total	17.67	

Table 6. Truthfully labelled (TFL) seed production and distribution of salinity and water logged tolerant rice varieties.

* For water logged areas

Noni: A wonder crop for saline and water logged soils

Noni (Morinda citrifolia L.) or Indian mulberry (Rubiaceae) with over 200 bioactive compounds for human wellness (relives of cancer, infection, arthritis, diabetes, asthma, pain etc. issues (Senthilkumar et al., 2016), believed to have originated in ANI and Indonesia was found ideal tree crop for both saline and waterlogged areas (Figure 12a & 12b) including brackish waters. Its cultivation in coastal states of India (286 ha at present) especially concentrated in ANI and Maharashtra (Marimuthu and Peter, 2010) was gradually picking up. It was thus a best bet for not only tsunami affected areas but also for climate change induced sea level rise areas. Establishment of noni based product line factory in ANI was a welcome step in its further wider exploitation. Release of four varieties (CARI Sampada, CARI Rakshak, CARI Samridhi and CARI Sanjivini) by ICAR-CIARI, standardisation of its production technology (Singh et al., 2011) including processing (Swain et al., 2018) paved way for its wider cultivation. Its suitability for intercropping under coconut plantations creates a new niche for its integration in coastal regions. Acceptance of noni foliage (Pascual et al., 2011) and fruit directly as therapeutic feed of pig (Sunder et al., 2016) will diversify the pig feed (copra and grazing) offered by Car Nicobar district tribal's of ANI for it was a pet animal. The utility of application of dye prepared from Noni mixed with garlic extract to trees for termite management also shows its wider utility avenues. It can also be used as feed for silkworms (Senthilkumar et al., 2016). Its utility as windbreak vegetation, shade tree for coffee and support for trailing pepper vines are additional areas for exploitation in the country.



Fig.12a Noni fruit at CIARI, Port Balir farm Fig. 12b Noni in saline and water logged (Source: Dr. B. Gangaiah, ICAR-CIARI)

conidtion at Sippighat, Port Balir (Source: Dr. B. Gangaiah, ICAR-CIARI)

6.7. **Raft Culture of Vegetables**

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Backwater lowlands stagnated with rain water like ponds were put to vegetable cultivation by soil less culture techniques life rafts. Rafts made of bamboo were prepared on air filled tubes (that keeps the unit afloat over water). A plastic sheet was spread on the raft for spreading the growth media (coco peat, saw dust and FYM) for 8-10 cm thickness (Figure 13a). Coriander / Dhania were seeded in the bed at NICRA-CGP project site at Ograbraj, South Andaman district on 16th October, 2018 (Figure 13b). Other high value vegetables like pudina, spinach, broccoli could also be cultivated.



Fig. 13a Bamboo raft made to float on tubes Fig. 13b Growth meadi filled raft sedded (Source: B. Gangaiah, ICAR-CIARI, Port with corinader (Source: B. Gangaiah, ICAR-Blair) CIARI, Port Blair) Poultry and Duckery Interventions in Water Logged Areas



Poultry shed made above backwater level



Start of poultry unit



Poultry at 7th week stage

Ducks at 6th week age

Fig. 14 Poultry and duckery intervention at Sh. Abdul Kadar, tsunami at Ograbraj, South Andaman

6.8. Land Shaping Technologies for Addressing Hydrological and Chemical (Salinity)

Poultry and duckery units were established in tsunami water logged areas by constructing shed above the water level (Figure 14). 'Vanaraja' poultry birds (100 bird unit) that command a premium price in the market (Rs. 200/kg live bird weight) were reared broiler purpose for 7 weeks (20th July- 10th September, 2018) achieving a live bird weight of 1.60 kg with a feed conversion ratio of 2.17 and net income of Rs. 7595. Duck (a water loving bird) rearing (50 bird unit) was started in semi-intensive production mode (day time grazing in tsunami water fields with concentrates feeding in sheds during night) in last week of August, 2018. The birds in their 6th week have attained a live weight of 750 g on 15th October. These farmer's participatory technologies tested with full financial support from NICRA-CGP project were replicated with more farmers on 50% cost sharing basis (Gangaiah, 2018).



7. Constraints

Land shaping was an engineering intervention for overcoming hydrological (water stagnation) and salt problems of farming regions through rearrangement of soil. In this approach, from waterlogged and saline areas, soil was dug to create a furrow /pond and the dugout soil was arranged on all sides to form a raised bed. A diagrammatic representation (Gupta *et al.*, 2006) was given in Figure 15 to depict the details. For creation of broad bed and furrow (BBF) system an expenditure of Rs. 1, 92, 350/ha (at 2013-14 prices) was required for ANI (Mandal *et al.*, 2015) and farming on BBF with vegetables etc. and rice –fish system have enhanced the system profits to Rs. 2,12,501 and 1,47,991 when compared to no land shaping intervention (Rs. 24000/ha). Collection of huge quantity of water in ponds and furrows may facilitate post-monsoon cropping. Elevated portions of bunds of BBF system lying above water levels gets removed of salts better than no intervention (Velmurugan *et al.*, 2015) that resulted in higher soil pH values of BBF than no intervention (Table 7). Under ICAR-NAIP project, over 200 BBF were made in ANI that successfully diversified the lowland rice based farming system (Ambast *et al.*, 2010).



Fig. 15 Schematic diagram of broad bed and furrow (BBF)

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Parameter	Immediately after tsunami (February, 2005)	After 8 years (March 2013)	
		Under BBF	No intervention
pHs	7.24a	6.36 b	5.88 с
ECe (dS/m)	22.14a	1.18 b	1.90 b
Na+ (meq/l)	173.2 а	33.2 с	55.4 b
Ca+2 (meq/l)	26.06 a	16.20 с	20.45 b
SAR	48.11 a	11.75 с	17.43 b
HCO3- (meq/l)	2.38 a	0.36 b	0.50 b
CI- (meq/I)	142.8 a	30.8 с	47.0 b
SO4-2 (meq/l)	62.4 a	14.4 с	33.6 b

Table 7. Effect of BBF and no intervention on soil properties in tsunami areas of ANI.

Different letter within row indicate a significant difference at p<0.05.

Coconut Plantation Development in Tsunami Lands

Coconut (Cocos nucifera) was the choice crop of coastal regions with high salinity tolerance but was too sensitive to water logging. Water stagnation cum salinity (water and soil from surface and sub-surface sea water entry) in tsunami impacted areas has ruined the coconut plantations leaving behind fronds without leaves. In some places even the fronds got killed leaving only roots and a foot of stump on which aquatic and saline tolerant vegetation has come up (Figure 16). The earthen embankment made also failed to provide protection from above degradation. In such area at Ograbraj, South Andaman owned by Malabar Coconut Cooperative Society, drainage lines were created through broad bed furrows (BBF) formation away from embankment on higher elevation under NICRA-CGP project. The subsurface saline water accumulated in furrows and on its beds; coconut plantation was done in July, 2018. On the compact block area (0.6 ha) created after BBF (3 number) coconut 'Andaman ordinary tall' block plantation was taken up. To generate disposable income to farmer, banana (China kela) suckers were planted an intercrop of coconut. Both the crops with 95-100% survival have got successfully established (Figure 17). The yields of banana in early 2019 and coconut in 5 years later is awaited (Gangaiah, 2018).



Fig. 16 Tsunami damaged coconut with only remnant fronds at Ograbraj, South Andaman district NICRA project site



Abandoned coconut land due to tsunami at start of intervention



Drainage channels making



Coconut + Banana + plantated on bed with fish in drainge channels

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Coconut + Banana block plantation done

Fig. 17 After agriculture restoration activities attempt in tsunami site of Figure 16

Livestock Interventions

Livestock are better components to saline and water logged environments than crops. Andaman & Nicobar Islands unique goat breed Teressa was known to survive on drinking salt waters and feeds that got registered with ICAR-NBARGR, Karnal as a breed (India_goat_3300_Teressa_06025) and this act was facilitated ICAR-CIARI. Looking at past survival records on salt water and feeds, and popularity with tsunami affected Nicobari tribal's, Teressa goat (a male and female goat) performance in tsunami affected lands under NICRA-CGP project (Figure 18) was started.



Teressa goat browsing in Tsunami lands

Fig. 18 Goatery intervention at the field of Sh. Abdul Kadar, Ograbraj.

Under NICRA - CGP project two BBFs were made (5 m wide furrow, 7 m wide bed; 40 m length) at Ograbraj, South Andaman to overcome water logging problem. On such bed, coconut was intercropped with banana, areca nut, spices and seasonal vegetables. In the pond, fish (tilapia) was introduced with crop wastes and poultry litter as feed. Vegetables (cowpea, bhendi) were harvested successfully for self consumption as well as marketing. Tapioca, elephant foot yam crops are at tuber formation stage



First day of Intevention

BBF making completed

Crops components added BBF

Fig. 19 BBF intervention site at Ograbraj, South Andaman field of Sh. Abdul Kadar

8. Conclusion

Several challenges are encountered in restoration of agriculture in earthquake ocean submerged, high tide sea water ingress regions and also in backwater lowlands impacted by salinity. The major challenge came from restrictive and unframed coastal land use regulations especially for aquaculture purpose in the Islands. The other problem emerged in sea vicinities was man –salt water crocodile (Crocodylus porosus)conflict that limited the use of beaches for tourism and coasts for aquaculture purposes.

Sea weed farming, shrimp culture, crab fattening etc. in completely submerged or high tide submerged lands and salinity / water logging coping mechanisms of land shaping and raft for backwaters lowland regions could aid in restoring agriculture. Re framing / changing land use regulation laws for coastal regions needs immediate attention of policy makers. The solutions evolved in 2004 earthquake impacted regions of Andaman and Nicobar holds good for climate change induced sea level rise to a major extent with the exception that the later has temperature component also that may lead to redistribution of biota.

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