

HERBICIDE TOLERANT RICE RESEARCH IN INDIA



MK Kar
M Chakraborti
S Munda
S Saha
P Swain
AK Mukherjee
M Behera
D Majhi
K Kumari
NP Mandal
S Samantaray
AK Nayak



ICAR-National Rice Research Institute
Cuttack-753006, Odisha, India



Herbicide Tolerant Rice Research in India

**MK Kar, M Chakraborti, S Munda, S Saha, P Swain,
AK Mukherjee, M Behera, D Majhi, K Kumari, NP Mandal,
S Samantaray and AK Nayak**



ICAR-National Rice Research Institute
Cuttack-753006, Odisha, India



Correct Citation

Kar MK, Chakraborti M, Munda S, Saha S, Swain P, Mukherjee AK, Behera M, Majhi D, Kumari K, Mandal NP, Samantaray S and Nayak AK. (2024). Herbicide tolerant rice research in India. NRRI Research Bulletin No. 51. ICAR-National Rice Research Institute, Cuttack, Odisha, 753006, India, pp.28.

Published by

Director
ICAR-National Rice Research Institute
Cuttack-753006, Odisha, India

August 2024

Disclaimer

ICAR-National Rice Research Institute is not liable for any loss arising due to improper interpretation of the scientific information provided in the research bulletin.

Printed in India at Print-Tech Offset Pvt. Ltd.,
Bhubaneswar, Odisha 751 024.



डॉ. हिमांशु पाठक

DR. HIMANSHU PATHAK

सचिव (डेयर) एवं महानिदेशक (आईसीएआर)

Secretary (DARE) &
Director General (ICAR)

भारत सरकार
कृषि अनुसंधान और शिक्षा विभाग एवं
भारतीय कृषि अनुसंधान परिषद
कृषि एवं किसान कल्याण मंत्रालय, कृषि भवन, नई दिल्ली-110 001

GOVERNMENT OF INDIA
DEPARTMENT OF AGRICULTURAL RESEARCH AND EDUCATION (DARE)
AND

INDIAN COUNCIL OF AGRICULTURAL RESEARCH (ICAR)
MINISTRY OF AGRICULTURE AND FARMERS WELFARE
Krishi Bhavan, New Delhi 110 001

Tel: 23382629 / 23386711 Fax: 91-11-23384773

E-mail: dg.icar@nic.in

FOREWARD

Direct seeded rice (DSR) cultivation is gaining popularity as it saves water, labour, time and even protects the environment by reducing the emission of greenhouse gases responsible for global warming. However, high weed infestation and evolution of weedy rice create a major hurdle for this type of rice cultivation. There is no chemical which can effectively control weedy rice without damaging the cultivated rice as they are very much similar to each other. This problem can be effectively managed by the cultivation of herbicide tolerant rice varieties in combination with application of herbicides. In recent years, researchers in our country have identified herbicide (Imazethapyr) tolerant mutant line 'Robin' in the mutant population of N22. The mutant line has been thoroughly characterized and the underlying genetic mechanism conferring herbicide tolerance has been deciphered. Many herbicide tolerant varieties have been released in our country utilizing this source.

ICAR-NRRI, Cuttack has recently developed the herbicide tolerant rice variety CR Dhan 807 in the background of popular upland rice variety 'Sahbhagidhan' through marker assisted backcross breeding. This has been released and notified for six states namely Jharkhand, Odisha, Chhattisgarh, Gujarat, Andhra Pradesh and Tamil Nadu under rainfed direct seeded upland condition. The variety specially addresses the concerns of high cost of weed management of small and marginal farmers in India.

This research bulletin on "**Herbicide tolerant rice research in India**" published by ICAR- National Rice Research Institute, Cuttack highlights the development of herbicide tolerant rice in the country and the economic and environmental benefits of growing HT rice. I wish this bulletin will serve as a valuable resource providing insights into the herbicide tolerant rice research in India.

(Himanshu Pathak)

Dated the 19th August, 2024
New Delhi

PREFACE

Rice is traditionally grown under transplanted condition after repeated puddling, which is not only time-consuming but also labour as well as water intensive. Increasing water scarcity due to climate change and unavailability of labour at the crucial stages of land preparation, transplanting and weeding is making this widely adopted method of rice production unsustainable.

One of the potential solution to address these challenges faced by the rice growers is direct seeded rice (DSR). Direct seeded rice can be used as a sustainable, and economically viable rice production system as it saves water, labour, time and even protects the environment by reducing the emission of greenhouse gases responsible for global warming. However, weeds and weedy rice create a major hurdle for this type of rice cultivation, and they compete with rice plants for water, nutrients, space, and sunlight. Weedy rice is an emerging threat which infests the direct seeded rice fields which are morphologically like cultivated rice in vegetative stage and can only be identified when it flowers. Hence, its control is not possible even by manual weeding during vegetative stage. At the same time, any herbicide which can't kill rice plants will also not be able to control weedy rice. In the case of Herbicide Tolerant (HT) rice, the application of specialized herbicides kill the weedy rice at vegetative stage without hindering the growth of rice.

In India, a mutant line (HTM-N22, later named as 'Robin') was identified under the N22 Functional Genomics Project, which was able to tolerate the herbicide Imazethapyr without any major phytotoxic effect due to mutation in Acetohydroxy Acid Synthase (AHAS) gene. This mutant line was further utilized to develop herbicide tolerant versions of popular non-basmati and basmati rice varieties developed by public sector in India. At ICAR-National Rice Research Institute, Cuttack, this gene has been transferred to four popular varieties, namely Sahbhagidhan (for uplands), Naveen (for favorable uplands and irrigated), Swarna Sub1 and Pooja (both for shallow lowlands). The herbicide tolerant Near Isogenic Line (NIL) of Sahbhagidhan has been released as CR Dhan 807 which is the first non-GM HT rice released in the country in non-basmati category. This variety is not only suitable for dry direct seeded rainfed upland condition but also for the zero tillage direct seeded resource conservation practices.

This bulletin presents the latest advancements made by the ICAR-National Rice Research Institute (ICAR-NRRI) in developing this herbicide tolerant rice. This bulletin serves as a valuable resource for researchers, agronomists, extension workers, and farmers, providing insights into the herbicide tolerant rice cultivation in India.

AUTHORS

CONTENTS

Sl. No.	Title	Page No.
1.	Introduction	6
2.	Herbicide tolerance in rice	8
3.	Herbicide Tolerant (HT) rice, a solution for managing weedy rice	10
4.	Herbicide tolerant rice cultivars, a global scenario	10
5.	Development of herbicide tolerant (HT) rice in India	11
6.	CR Dhan 807: India's first non-GM herbicide tolerant non-basmati rice variety	15
7.	Package of practices for HT rice cultivar CR Dhan 807	19
8.	Dos and Don'ts for cultivation of HT-rice	22
9.	Economic and environmental benefits	23
10.	Way forward	24
11.	References	25

1. Introduction

In India, rice is considered as a major crop which is grown in almost all the states. Majority of the farmers in the country grow rice through transplanting method which is labour as well as water intensive. Transplanting of rice in puddled soils reduce the weeds in rice field if stagnant water is maintained. To get one kg of rice, nearly 2500 litres of water is needed from about 30 irrigations (Bouman, 2009). However, with increasing scarcity of water, it is becoming impossible for many farmers to maintain stagnant water throughout the crop period and at many times, the fields remain dry or with saturated water only. Under such situations, weeds also grow quite vigorously even in transplanted paddy fields. The cost of weed management through manual operations can reach to even 30% depending on the growing conditions (Rao et al.2015). In last two decades, with rapid urbanization and changing socioeconomic scenarios, migration of labourers to other states from the rural areas of eastern India enhanced significantly and thus the paddy farmers regularly face the problems of labour shortage and finally pay higher cost for the same work in a unit area. To reduce cost of transplanting and save water, many farmers are avoiding transplanting and adopting direct seeding where dry (in dry fields) or pre-germinated (in wet fields) seeds are directly sown into the field, thereby cutting the cost of nursery preparation as well. Direct seeded rice cultivation saves water, labour, time and even protects the environment by reducing the emission of greenhouse gases responsible for global warming. However, weeds create a major hurdle for this type of rice cultivation, and they compete with rice plants for all the natural and artificial inputs required in cultivation ranging from sunlight to fertilizers applied. Nearly 18–48% yield loss can happen due to weed infestation alone in rice (Rao et al.2007). The plants also become weak and succumb to the attack of different insects and pathogens. The quality of the produce also gets adversely affected. With the advent of DSR, apparently unfamiliar weeds to rice such as *Echinochloa crusgalli* and *Leptochloa chinensis*, started appearing in rice fields nearly 40 years ago (Azmi et al. 2005). In subsequent years, other weed species like *E. crusgalli*, *Commelina diffusa* L., and *Cyperus rotundus* L. gradually became

predominant (Singh et al. 2005, Yaduraju and Mishra, 2008). Besides India, predominance of sedges under DSR cultivation have been recorded in countries like Australia, the USA (Gressel, 2002) and Malaysia (Azmi and Mashhor, 1995).

Weeds do not add to economic harvest but draw nutrients, water etc. from the same field and thereby reduce the yield of target crops. Farmers manage the weeds in rice through combination of methods like herbicide application, tillage, puddling, mulching, crop rotation, irrigation, mechanical weeding, and hand weeding. With increased cost of farm labourers, manual weeding is becoming very costly for the farmers. More and more farmers are looking for alternatives and application of herbicides is becoming popular for weed management in rice. In general, herbicides kill most of the undesirable plants present in the fields. If a recommended herbicide is applied at appropriate dose and suitable plant growth stages, the weeds get killed and no adverse effect is observed on the crop grown there. The most widely used herbicides in rice are penoxsulam, cyhalofop-butyl, fenoxaprop, pretilachlor, imidazolinone, bensulfuron, carfentrazone, sulfonyleurea, molinate, bentazone, clomazone, pyrazosulfuron, propanil, bispyribac sodium and pyrimidinyl-benzoate (Rao et al.2007; Bai et al.2017). These herbicides can control the weeds of rice and their applications helped in improving the productivity. However, weedy rice is an emerging threat which infests the DSR fields and can not be controlled by these selective herbicides. Weedy rice belongs to the genus *Oryza* and is taxonomically not classified as a distinct species from *O. sativa*. Weedy rice is considered as the natural progenitors derived from exchange of genomic segments between wild and cultivated rice species (belonging to AA genome) through spontaneous hybridization in crop fields. Due to their genetic and morphological similarity with cultivated rice, weedy rice is not affected by selective herbicides used in rice cultivation. At the same time, competitive ability of weedy rice is very high, and their presence poses major challenges for DSR. Hence, only the herbicides that will kill the rice crop can control the weedy rice. There is need for developing herbicide tolerant rice varieties where selective control of both weedy rice and other weed flora can be made possible to sustain the DSR as economically viable alternative.

2. Herbicide tolerance in rice

There are certain herbicides like pretilachlor, bispyribac sodium etc. which can control weeds of rice to a certain extent but do not cause major phytotoxicity to the rice plants. On the contrary, there are large number of herbicides that kill the rice crops upon application. Generally, such herbicides disrupt the key metabolic processes regulating growth and development of rice plants. The physiological and biochemical mechanisms have been well studied for several widely used herbicides like glyphosate, glufosinate, synthetic auxins, sulfonylurea, imidazolinones, triketones, isoxazoles, callistemone, cyclohexanediones, aryloxyphenoxypropionates and phenylpyrazolines. At the same time, new herbicide molecules also regularly come to market with their unique mode of actions. The knowledge generated from those studies further helped to develop herbicide tolerant genotypes. Mostly, the transgenic route was followed, besides mutation breeding in few other cases (Endo and Toki, 2013). Among the herbicides, Glyphosate remained the major target considering its total-killing broad-spectrum properties.

Table 1: Transgenic herbicide tolerant rice developed worldwide

Transgene introduced	Tolerance against herbicide	Genetic background	Reference
Human cytochrome P450 genes <i>CYP1A1</i> , <i>CYP2B6</i> and <i>CYP2C19</i>	Pyributicarb, Acelachlor, Metolachlor, Thenylchlor, Chlorotoluron and Norflurazon	-	Kawahigashi et al. (2004)
G6 <i>EPSPS</i> derived from the bacteria <i>Pseudomonas putida</i>	Glyphosate	Xiushui 110	Zhao et al. (2011)
Codon-optimized P4 <i>EPSPS</i> gene derived from <i>Petunia hybrida</i>	Glyphosate	IR64	Chhapekar et al. (2016)
Combination of <i>EPSPS</i> encoding genes from the bacteria <i>Janibacter</i> sp.	Glyphosate	Zhonghua 11 (<i>japonica</i> rice)	Cui et al. (2016)
<i>I. variabilis EPSPS</i> gene from the bacterial <i>Isoptericolavariabilis</i>	Glyphosate	Minghui 86	Yi et al. (2016)

<i>OsmEPSPS</i> (mutant gene of rice) + <i>igrA</i> gene from bacteria <i>Pseudomonas</i> sp.	Glyphosate	Swarna	Fartayal et al. (2018)
<i>bar</i> gene	Glufosinate	Gulfmont, IR72, and Koshihikari	Orad et al. (1996)
		Xiushui 04, Minghui 63, R187, D68, and E32	Xiao et al. (2007, 2009)
		Upland rice lines 297 and 502	Tian et al. (2015)
<i>RePAT</i> gene from the bacteria <i>Rhodococcus</i> sp.	Glufosinate	Zhonghua 11	Cui et al. (2016)

Besides transgenic route, modern tool of genome editing created unique opportunity to induce mutations in rice genes and several tolerant lines have been developed worldwide.

Table 2: Genome editing based herbicide tolerant rice developed worldwide

Target gene edited	Gene editor	Tolerance against herbicide	Reference
EPSPS	CRISPR/Cas9	Glyphosate	Li et al. (2016)
ALS	CRISPR/Cas9	Bispyribac sodium	Sun et al. (2016)
	Target-AID	Imazamox	Shimatani et al. (2018)
	BEMGE	Bispyribac sodium	Zhang et al. (2020)
TubA2	rBE14	Pendimethalin, trifluralin	Liu et al. (2020)
ACCase	STEMEs	Haloxypop	Li et al. (2020)
	eABE, eBE3	Haloxypop-R-methyl	Liu et al. (2020)

At present, the transgenic or genetically modified (GM) crops are under strict regulatory norms in most countries and even not allowed for cultivation in many others. Non-GM herbicide tolerant crops, on the contrary, are widely accepted.

Among the different herbicides that kill rice plants, imidazolinones have been targeted to develop herbicide tolerant rice varieties. The herbicide group disrupts Acetolactate Synthase (ALS) or Acetohydroxy Acid Synthase (AHAS) enzymes. The inhibition of these enzymes prevents synthesis of branched amino acids like valine, leucine and isoleucine which are highly essential for survival of rice plants (Singh and Shaner, 1995). Specific mutations in the *AHAS* gene involved in the synthesis of Acetohydroxy Acid Synthase enzyme can alter the binding site for imidazolines and confer tolerance to imidazolinones.

3. Herbicide Tolerant (HT) rice, a solution for managing weedy rice

In several parts of eastern, northeastern and southern India, weedy rice creates major hurdle in cultivation of paddy especially under direct seeded conditions. Weedy rice originates from spontaneous natural outcrossing between cultivated rice and its wild relatives. These are generally awned with shattering habits besides having strong seed dormancy. If unchecked, these plants enhance their population substantially within a short span of time in the rice fields and compete with the rice crop and reduce the yield levels. Unfortunately, weedy rice is morphologically similar to cultivated rice in vegetative stage and can only be identified when they flower. Hence, its control is not possible even by manual weeding during vegetative stage. At the same time, any herbicide which cannot kill rice plants will also not be able to control weedy rice. In case of HT-rice, Imazethapyr application kills the weedy rice at vegetative stage without affecting the rice crop.

4. Herbicide tolerant rice cultivars, a global scenario

In general, the traditional herbicides applied in rice crop at their respective recommended doses do not cause major phytotoxicity to the rice plants. Till 2021, herbicide tolerant rice varieties were not available in India. In other countries, few herbicide tolerant rice varieties were released (like Liberty Link, Provisia, Clearfield, and Jietian). Liberty Link (developed by Aventis) and Roundup Ready (developed by Monsanto) are cultivated in the USA specifically to control the weedy rice (red rice). Liberty Link rice can tolerate the applications of glufosinate whereas Roundup Ready rice varieties withstand applications of glyphosate, both of which are broad spectrum non-selective herbicide. The Liberty Link rice varieties, LLRICE 06 and LLRICE 62 were developed through transgenic route

through transformation of the *pat* gene derived from a bacteria *Streptomyces hygroscopicus*. Liberty Link rice shows tolerance to the herbicide glufosinate and was approved for food or feed in the USA during the years 2000 followed by Canada in 2006 (Quirasco et al., 2008). LLRICE 62 was also approved for consumption in Mexico, Russia, Australia, New Zealand, and Honduras. Louisiana State University Agricultural Center developed another transgenic rice variety showing tolerance to herbicide Quizalofop which is an acetyl coenzyme A carboxylase inhibitor. This GM-HT rice was named Provisia and the first variety PVL01 (long grain rice) of this series was approved for release and commercial cultivation in 2017 (Famoso et al. 2019).

In the non-GMO category, the first varietal category was the ‘Clearfield’ rice developed by Louisiana State University Agricultural Center and American Cyanamid- BASF through induced mutagenesis in the endogenous *ALS* (acetolactate synthase) gene of rice. Clearfield rice can tolerate imidazolinone herbicides effective against broad spectrum weeds. It was first commercialized in the United States in 2002 (Jin et al.2022). In Malaysia, imidazolinone tolerant rice varieties, MR220CL1 and MR220CL2 were developed by the ‘Clearfield’ production system and was commercially introduced in 2010 (Ruzmi et al.2021). Subsequently, six Jietian rice varieties carrying the Trp548Met mutation in *ALS* gene were released in China (Chen et al.2020). These varieties were developed through chemical mutagenesis using ethyl methane sulfonate and the mutations confer tolerance to the herbicide Imazethapyr. Unfortunately, all the GM and non-GM herbicide tolerant lines or genetic stocks developed elsewhere are protected through patents/IPR (Croughan 1998, 2002; Livore 2003) and is not available for public sector research institutes in India for use in rice breeding.

5. Development of herbicide tolerant (HT) rice in India

Like the global scientific community, Indian scientists also attempted to develop transgenic glyphosate tolerant rice varieties in which they were successful. At ICAR-NIPB, transgenic IR64 with codon-optimized P4 *EPSPS* gene derived from *Petunia hybrida* was used for developing glyphosate tolerant lines (Chhapekar et al.2016). At International Centre for Genetic Engineering and Biotechnology, New Delhi, glyphosate tolerant rice was developed in the background of popular variety Swarna by developing transgenic plants with *OsmEPSPS* and *igrA* genes

(Fartayal et al.2018). Both the attempts were taken through the approach of GM crop and were not subsequently released for cultivation.

Simultaneously, the scientists in India tried to explore the non-GM route through mutagenesis. ICAR-NIPB developed several EMS mutant lines of upland genotype N22 (Mohapatra et al.2014) which were further evaluated for herbicide tolerance at Tamil Nadu Agriculture University in a consortium project funded by department of Biotechnology, Government of India involving ICAR-NIPB, ICAR-IARI, ICAR-NRRI, ICAR-IIRR, TNAU (Coimbatore) and UAS (Bengaluru). The project successfully identified a mutant line (HTM-N22) which was able to tolerate the herbicide Imazethapyr (Shoba et al.2017). In general, rice plants cannot tolerate the herbicide Imazethapyr and 100% mortality is observed when sprayed as 10% SL. The mutant line later named as ‘Robin’ can tolerate the herbicide without any major phytotoxic effect due to mutation in Acetohydroxy Acid Synthase (*AHAS*) gene. Five unique SNPs were identified in the *AHAS* gene of the mutant line Robin compared to N22. However, which individual SNP or combination of SNPs of the gene confer Imazethapyr tolerance is not known with certainty. A SNP/mutation at the 152nd amino acid has been putatively identified as the causal mutation in the Robin mutant. The mutations in the independently developed line Robin were found to be unique in nature and can be utilized without any IPR infringements among the partner institutes to develop commercial varieties.

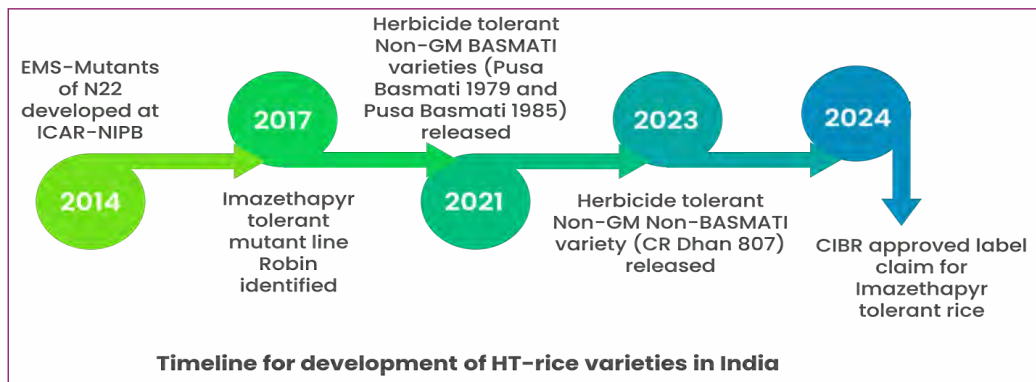


Fig. 1. Timeline for development of non-GM herbicide tolerant rice in India

This mutant line was further utilized to develop herbicide tolerant versions of popular non-basmati and basmati rice varieties from the public sector in India. During development of these varieties, introgression of foreign genes was not attempted at any stage and the products do not belong to the category of genetically modified organisms (GMO). The varieties with herbicide tolerance traits have been extensively tested under All India Coordinated Research Project on rice for their agronomic efficacy and subsequently notified by Central sub-Committee for Crop Standards, Notification and Release of Varieties. Further, extensive studies were conducted on the residue levels of Imazethapyr and the other safety issues for environment and foods and based on those, the Imazethapyr tolerant rice varieties finally received clearance for general cultivation as direct seeded rice (DSR) coupled with Imazethapyr application from *Kharif* 2024 onwards in India from the Central Insecticides Board & Registration Committee. While the HT-rice varieties strive Imazethapyr application, majority of the weeds especially grasses and sedges besides the weedy rice found in the rice fields are effectively controlled. This technology enables complete mechanization of rice as a practicable option and address the major issues of labour scarcity and increased cost of cultivation of rice in the country. Cultivation of HT-rice through DSR can substantially enhance the profitability of paddy cultivation. All HT-rice varieties developed from the Robin mutant is identified with the trademark of **RobiNOweed**.

Pusa Basmati 1979 and Pusa Basmati 1985 were developed by Indian Agricultural Research Institute for the areas of basmati cultivation. These varieties are the country's first ever non-genetically modified (non-GM) herbicide tolerant basmati rice varieties which confers resistance to Imazethapyr. It was developed by backcrossing two popular varieties, Pusa 1121 and Pusa 1509 with Robin. Under All India Coordinated Rice Improvement Project (AICRIP), the varieties were tested for their agronomic performances and effective weed control was obtained from only one spray. At ICAR-National Rice Research Institute, Cuttack, this gene has been transferred to four popular varieties, namely Sahbhagidhan (for uplands), Naveen (for favorable uplands and irrigated), Swarna *Sub1* and Pooja (both for shallow lowlands). The herbicide tolerant line of Sahbhagidhan has been released as CR Dhan 807 which is the first non-GM HT rice released in the country in non-basmati category. Several other popular non-basmati varieties are also being improved in India for the trait.

Table 3. HT-rice cultivars currently under development in ICAR-NARES from the mutant line Robin/its derivatives

Cultivar backgrounds (non-basmati)	Institute involved
Naveen, Swarna, Pooja, CR Dhan 312 and additionally >100 crosses being advanced through speed breeding	ICAR-NRRI, Cuttack
Pusa 44	ICAR-IARI, New Delhi
DRR Dhan 42 (IR64 Drt1)	ICAR-IIRR, Hyderabad
Co51, Anna 4 and ADT 43	TNAU, Coimbatore



**Weed population
(before Imazethapyr application)**



**Effective weed management
(12 days after Imazethapyr application)**

Fig. 2. Herbicide (Imazethapyr) tolerant rice provides cheaper alternative for efficient weed management in rice

6. CR Dhan 807: India's first non-GM herbicide tolerant non-basmati rice variety

CR Dhan 807 (CR4333-35-2-2-1/IET30438) is a non-GM herbicide (Imazethapyr) tolerant near isogenic line (NIL) of popular variety Sahbhagidhan grown in several rice growing states of India. As the recurrent parent is already popular among farmers, consumers and industry, the NIL can be accepted easily by the stakeholders. The HT-NIL of Sahbhagidhan (CR Dhan 807) has been released for six states namely Jharkhand, Odisha, Chhattisgarh, Gujarat, Andhra Pradesh and Tamil Nadu. The variety specially addresses the concerns of small and marginal farmers in India.



Fig. 3. Aerial picture depicting herbicide tolerant versions of popular rice varieties vis-à-vis their susceptible versions after spray of Imazethapyr.



Fig. 4. Field view of CR Dhan 807 grown till maturity after herbicide application



Fig. 5: Appearance of grains of recurrent parent Sahbhagidhan, donor parent Robin and HT-NIL CR Dhan 807

CR Dhan 807 has been developed using marker assisted backcross breeding (MABB) strategy where the mutant line Robin was used as donor. Three backcrosses were performed with recurrent parent, Sahbhagidhan to recover >95% of its genome. The key features of the variety are

- This variety matures within 110-115 days and is suitable for growing under rainfed direct seeded upland conditions where weed and weedy rice management are major problems.
- Besides dry DSR, the variety is also suitable for zero tillage-DSR cultivation.
- Under rainfed direct seeded trials, the variety showed average yield potential of 4.4 t/ha under normal rainfall across all test locations of the country and 2.8 t/ha under moderate drought situations. Yield potential up to 6.8/ha was recorded under normal rainfall in AICRIP trials.
- Imazethapyr application do not cause any phytotoxicity effect in CR Dhan 807 but effectively controls the weeds when applied at appropriate stage.
- No yield penalty from Imazethapyr application was recorded when compared with plots which were kept weed free through manual weeding.
- Besides herbicide tolerance, the variety also possesses better weed competitive ability due to its higher early seedling vigour.
- The variety CR Dhan 807 is also tolerant to drought and nutrient use efficient. This provides substantial advantage under rainfed situations.
- The variety will enable complete mechanization of direct seeded rice cultivation and address the major issues of labour scarcity and increased cost of cultivation in the country.
- DSR cultivation can also reduce water usage and greenhouse gas emission from rice fields. The variety can be grown efficiently under dry DSR without the requirement of nursery preparation or transplanting.

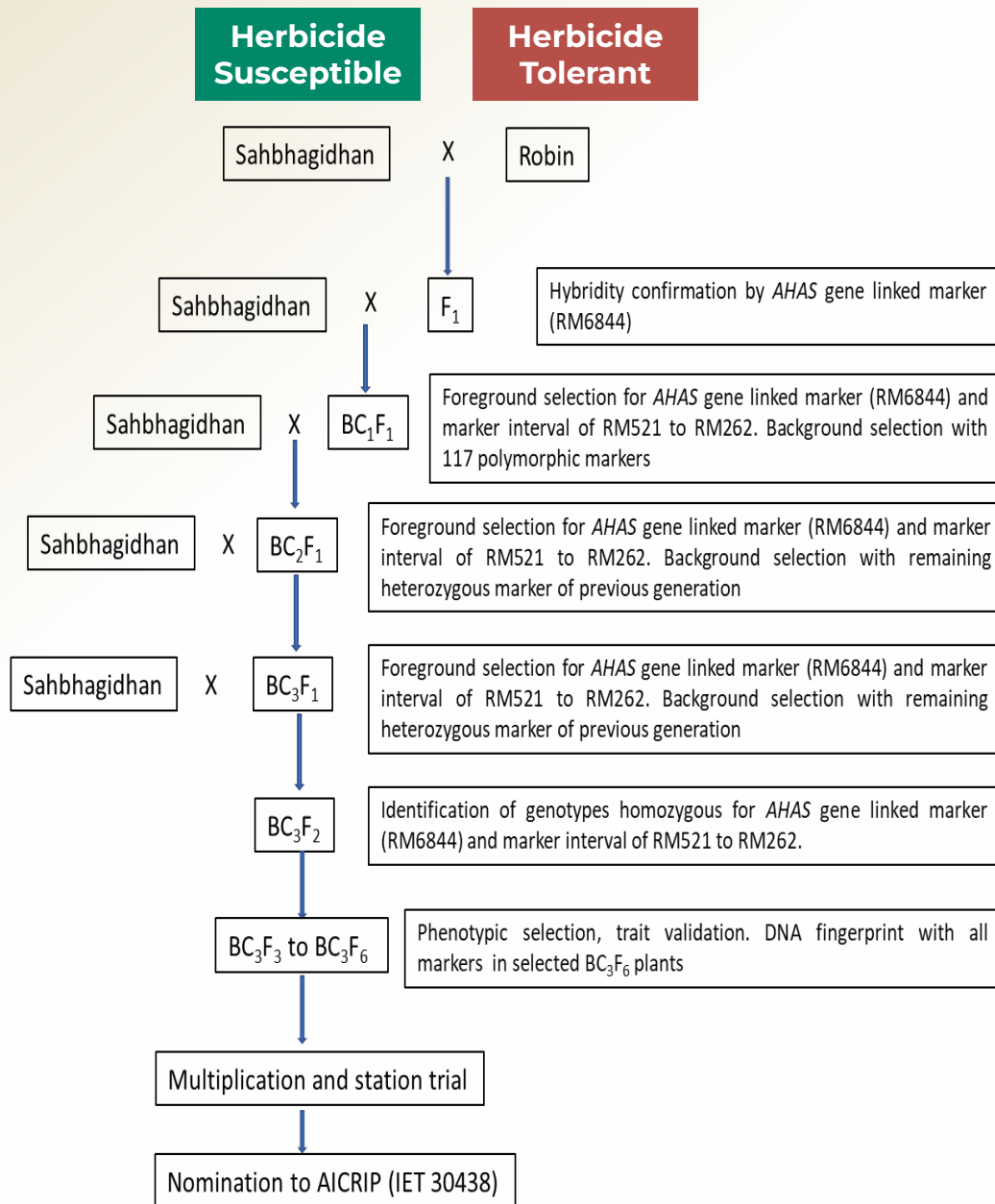


Fig. 6. Breeding scheme followed for development of CR Dhan 807

7. Package of practices for HT rice cultivar CR Dhan 807

The herbicide (Imazethapyr) tolerant rice variety CR Dhan 807 is suitable for cultivation under dry direct seeded and zero tillage-direct seeded condition in rainfed ecology.

- a) **Field selection:** Fields with good drainage facility to be selected. Both rainfed or favourable uplands are suitable for the variety.
- b) **Land preparation:**
 - i) **For dry direct seeded condition:** Prepare the land well using a rotavator or cultivator to get a fine tilth. Remove the weeds and crop stubbles before proper levelling for uniform germination and crop stand. Apply and incorporate 5t/ha of FYM / compost during dry ploughing. In areas with history of heavy weed infestation, growers can adopt stale seedbed technique by allowing weed seeds to emerge and then kill either by shallow tillage or by spraying Pendimethalin (1.0 kg ai/ha) during sowing as pre-emergence herbicide.
 - ii) **For zero tillage-direct seeded condition:** No land preparation is required for sowing. However, stale bed technique for controlling the weeds is a mandatory requirement. For stale bed apply Glyphosate @ 1200 g ai/ha two times before sowing. Last application is done 7 days before sowing. Soil should be saturated each time before spraying of herbicide to effectively kill the weeds. Moist soil also helps the escaped weeds/weed seeds to regenerate or germinate which can be controlled by the subsequent spray of herbicide.
- c) **Seed treatment:** Treat the seed with Carbendazim 50% WP @ 2g/kg seed before sowing.
- d) **Sowing time:** Wet season (*Kharif*) is recommended for growing the variety CR Dhan 807. Sowing should be done in situations where heavy rain is not expected in next 3-4 days.
- e) **Sowing method:** Use seed rate of 35-40 kg/ha for dry DSR and 45 kg/ha for ZT-DSR. Sowing can be done manually or by seed drill at 20 cm row to row distance to ensure better crop stand and canopy coverage.

- f) **Weed control measures:** Spray herbicide Imazethapyr @ 100 g a.i./ha i.e. 2.5 ml of commercial formulation (10% SL) per liter of water as foliar spray. Additionally, add 2.5 ml adjuvant in the solution. Spray by flat fan or flood jet nozzle within 15-17 days after seedling emergence gives satisfactory results. Spray the canopies properly covering complete surfaces. Weeds should be at least 2-3 leaf stages at the time of spray. Moisture should be sufficient in soil without any standing water and no irrigation should be given up to 48 hrs after herbicide application. If irrigation is available, the field may be irrigated/ ponded 48 hours after spray. The spray module of 15-17 DAE is specifically recommended for CR Dhan 807 only. The recommendation may vary with maturity duration of the other varieties, their inbuilt weed competitiveness, pattern of early growth and vigor etc.

The Imazethapyr application method remains the same in case of ZT-DSR also. The application must be completed within 19-20 after emergence. Late application is generally not desirable.

- g) **Fertilizer applications:** Apply NPK @ 60:30:30 kg/ha. Apply one-third of the nitrogen, entire amount of P and two-third of K as basal. Remaining amount of nitrogen may be given in two equal splits during tillering and booting stage. Remaining one-third of K is to be applied at maximum tillering. Additionally, a spray of 2% DAP at 3-4 days after herbicide application is desirable. If same sprayer is used, clean the nozzles thoroughly using water before application.
- h) **Irrigation:** May be grown as complete rainfed crop. However, if there is prolonged period without rain, need based supplementary irrigations will provide better yield.
- i) **Disease and pest control:** Protect the crop from insect pest and diseases with regular monitoring of pest attacks and by following need-based pesticide application. While spraying pesticide, use 500 liters of water/ha. Keep the field and field bund clean to minimize disease and pest attack. Application of Chlorantraniliprole (0.4% GR) @ 10 kg/ ha at brood emergence is very effective in controlling YSB and leaf folder. Foliar spray of Imidachloprid 17.8 sl @ 100 ml ha⁻¹ or Pyrometrozine 300 g ha⁻¹ can be applied for brown plant hopper, WBPH, etc. only in case of infestation above ETL.

- j) **Harvesting:** After physiological maturity with grain moisture content 15% or less.
- k) **Intercropping:** Beside growing rice as sole crop, CR Dhan 807 provides an opportunity for rainfed-upland farmers to simultaneously grow rice and crops like late-maturing pigeon pea as intercrops and manage weeds with the common herbicide Imazethapyr. The crops have different rooting zones and thus don't compete to draw nutrients. The early duration rice variety CR Dhan 807 matures first and then the pigeon pea continue to grow with residual moisture. This unique opportunity with the HT rice variety can buffer farmers income and augment soil health.



8. Dos and Don'ts for cultivation of HT-rice

DOs

- Only certified seeds of CR Dhan 807 from agencies authorized by ICAR-NRRI with RobiNOweed and NRRI logo to be procured. Truthfully Labelled (TL) seeds can be procured only from the farm section of ICAR-NRRI, Cuttack and its regional stations located in Hazaribagh (Jharkhand), Naira (Andhra Pradesh) and Gerua (Assam).
- The rice variety CR Dhan 807 should be grown under direct seeded rice cultivation.
- Use the recommended dose of Imazethapyr 10% SL (from standard brands with label claim clearance of CIBR) for effective control of weeds. Prepare solutions as per guidance provided in SOP for respective brands.
- Imazethapyr is a post-emergence herbicide. Take up the spray of Imazethapyr at 2-3 leaf stage of the weeds (approx. 18-20 DAS or 15-17 DAE) for effective control.
- In areas with history of very high weed pressure, pre-emergence application of Pendimethalin followed by Imazethapyr within 18-20 days after Pendimethalin application is advised.
- Apply Imazethapyr uniformly across field to avoid escapes.
- Use all necessary protective equipment and protocols for application of Imazethapyr.

DON'Ts

- Never procure seeds of CR Dhan 807 from unauthorized sources. If a non-HT variety seed is sown and Imazethapyr is applied, there can be complete crop loss.
- The weed stage should not cross 2-3 leaf stage at the time of application of Imazethapyr for effective weed control.
- Don't mix/ grow non-HT varieties in the same field to avoid crop loss/ phytotoxicity.

- Avoid spraying Imazethapyr if the wind speed is high to avoid damage due to drift, on neighboring non-HT rice crop.
- Avoid using tank mix of other chemicals/ fertilizers with Imazethapyr.
- Don't spray in a flooded field; maintain soil saturation only.
- Don't use herbicide near water bodies.
- Don't irrigate the field immediately after spraying of Imazethapyr.
- Don't spray Imazethapyr, if heavy rain is expected within 3-4 hour after the spray.
- Without authorization from ICAR-NRRI, the seeds of this variety should not be produced/sold by any agency. Violation of this clause may draw legal action as per guidelines of ICAR.

9. Economic and environmental benefits

CR Dhan 807 can be grown as direct seeded rice under complete rainfed conditions. Multilocation trials over years have proven that the yield of the variety under normal rainfall is at par with the varieties of similar duration grown under transplanted conditions. However, the variety CR Dhan 807 can also perform well under moderate drought situations where most of the other varieties may fail completely. This resilience of CR Dhan 807 along with high yield potential provides a broader opportunity for small and marginal farmers. Besides, the herbicide tolerant rice will substantially reduce the cost of cultivation which in turn will enhance the income of the farmers. The variety is also nutrient use efficient and provides optimum yield at fertilizer dose of 60:30:30 Kg N:P:K as compared to 25-30% higher dose needed in transplanted rice. Except under extraordinary circumstances, the variety doesn't require any supplementary irrigation in a normal rainfall year in rainfed area of India and may grow well with only rainwater. Thus, irrigation cost, besides cost incurred on puddling, nursery preparation etc. are completely waived in this variety and weed management cost is reduced to tune of 70-75%.

In terms of environmental benefits, the cultivation of this variety will almost completely forego groundwater exploitation which is a major environmental concern of modern agriculture. Suitability for DSR cultivation and drought

tolerance of the variety assures that the crop may be grown without need for keeping constant stagnant water throughout growing seasons. This can help to substantially reduce greenhouse gas (GHG) emission from rice fields which as per several estimates is not less than 30% reduction.

Overall, at similar yield level compared to transplanted rice or aerobic rice of similar duration grown through irrigation, the herbicide tolerant rice CR Dhan 807 when grown under direct seeded condition can reduce cost of cultivation by 30% in normal rainfall years. In the years of drought, the resilience of CR Dhan 807 will provide substantial buffering to farmers as additional benefit.

10. WAY FORWARD

The following areas of research need to be strengthened for herbicide tolerant rice development programme.

- Transferring herbicide tolerance to other genetic backgrounds suitable for direct seeded condition.
- Combining herbicide tolerance with other important traits conferring biotic and abiotic stress tolerance, grain quality, nutrient use efficiency, preferable plant architecture etc. by marker-assisted selection.
- Identifying tolerant sources for other herbicides in the germplasm or mutant population, so that, the problem of weeds (especially weedy rice) can be effectively solved by the cultivation of herbicide tolerant rice in combination with corresponding herbicides. This will also give opportunity for herbicide rotation.

11. REFERENCES

- Azmi M, Mashhor M (1995) Weed succession from transplanting to direct-seeding method in Kemubu rice area, Malaysia. *Journal of Bioscience*. 6: 143-154.
- Azmi M, Chin DV, Vongsaroj P, Johnson DE (2005) Emerging issues in weed management of direct-seeded rice in Malaysia, Vietnam, and Thailand. In: Toriyama K, Heong KL, Hardy B (Eds.), *Rice Is Life: Scientific Perspectives for the 21st Century*, International Rice Research Institute, and Tsukuba (Japan): Japan International Research Center for Agricultural Sciences, Los Baños (Philippines). pp. 196-198.
- Bai YL, Gu LL (2017) The market and development of three types of pesticides on rice. *Modern Agrochemicals*. 16: 1-7.
- Bouman B (2009) How much water does rice use? *Rice Today*. 28-29.
- Chhapekar S, Raghavendrarao S, Pavan G, Ramakrishna C, Singh VK, Phanindra ML, Dhandapani G, Sreevathsa R, Kumar PA (2015) Transgenic rice expressing a codon-modified synthetic *CP4-EPSPS* confers tolerance to broad-spectrum herbicide, glyphosate. *Plant Cell Reports*. 34: 721-731. <https://doi.org/10.1007/s00299-014-1732-2>.
- Chen L, Gu G, Wang CX, Chen ZF, Yan W, Jin M, Xie G, Zhou JL, Deng XW, Tang XY (2020) Trp₅₄₈Met mutation of acetolactate synthase in rice confers resistance to a broad spectrum of ALS-inhibiting herbicides. *Crop J*. pp. 750-758
- Cui Y, Huang S, Liu Z, Yi S, Zhou F, Chen H, Lin Y (2016) Development of Novel Glyphosate-Tolerant Japonica Rice Lines: A Step Toward Commercial Release. *Frontiers in Plant Science*. 7:1218. doi: 10.3389/fpls.2016.01218.
- Cui Y, Liu Z, Li Y, Zhou F, Chen H, Lin Y (2016) Application of a novel phosphinothricin *N*-acetyltransferase (RePAT) gene in developing glufosinate-resistant rice. *Scientific Reports*. 6: 21259. <https://doi.org/10.1038/srep21259>.
- Croughan TP (1998) Herbicide Resistant Rice. US Patent. 5:773-704.
- Croughan TP (2002) Herbicide Resistant Rice, US Patent Application 20020019313
- Dayan FE (2019) Current status and future prospects in herbicide discovery. *Plants*. 8: 341.
- Endo M, Toki S (2013) Creation of herbicide-tolerant crops by gene targeting. *Journal of Pesticide Science*. 38(2):49-59.
- Famoso AN, Harrell DL, Groth DE, Webster EP, Oard JH, Zaunbrecher RE, Bearb KF, Conner CA, Guidry GJ, Angira B (2019) Registration of 'PVL01' rice. *Journal of Plant Registrations*. 13: 330-333.

- Fartyal D, Agarwal A, James D, Borphukan B, Ram B, Sheri V, Yadav R, Manna M, Varakumar P, Achary VMM, Reddy MK (2018) Co-expression of P173S Mutant Rice *EPSPS* and *igrA* Genes Results in Higher Glyphosate Tolerance in Transgenic Rice. *Frontiers in Plant Science*. 9:144. doi: 10.3389/fpls.2018.00144. PMID: 29487608; PMCID: PMC5816812.
- Gressel J (2002) Preventing, delaying and mitigating gene flow from crops: Rice as an example. In: *Proceedings of the 7th International Symposium on the Biosafety of Genetically Modified Organisms, China, Beijing*. pp. 59-77.
- Jin M, Chen L, Deng XW, Tang X (2022) Development of herbicide resistance genes and their application in rice. *The Crop Journal*. 10(1):26-35.
- Kawahigashi H, Hirose S, Inui H, Ohkawa H, Ohkawa Y (2004) Enhanced herbicide cross-tolerance in transgenic rice plants co-expressing human CYP1A1, CYP2B6, and CYP2C19. *Plant science*. 168(3):773-781.
- Li J, Meng XB, Zong Y, Chen KL, Zhang HW, Liu JX, Li J, Gao C (2016) Gene replacements and insertions in rice by intron targeting using CRISPR-Cas9. *Nature Plants*. 2:16139. doi: 10.1038/nplants.2016.139.
- Li C, Zhang R, Meng X, Chen S, Zong Y, Lu C, Qiu JL, Chen YH, Li J, Gao C (2020) Targeted, random mutagenesis of plant genes with dual cytosine and adeninebase editors. *Nature Biotechnology*. 38:875-882.
- Liu L, Kuang YJ, Yan F, Li SF, Ren B, Gosavi G, Spetz C, Li XJ, Wang XF, Zhou XP, Zhou HB (2020) Developing a novel artificial rice germplasm for dinitroanilineherbicide resistance by base editing of *OsTubA2*. *Plant Biotechnology Journal*. 19:5-7.
- Liu X, Qin R, Li J, Liao S, Shan T, Xu R, Wu D, Wei P (2020) A CRISPR-Cas9-mediated domain specific base editing screenables functional assessment of ACCase variants in rice. *Plant Biotechnology Journal*. 18:1845-1847.
- Livore AB (2003) Rice Plants Having Increased Tolerance to Imidazolinone Herbicides: International Application Published Under the Patent Cooperation Treaty (PCT).
- Mohapatra T, Robin S, Sarla N, Sheshahsayee M, Singh AK, Singh K, Singh NK, AmithaMithra SV, Sharma RP (2014) EMS Induced Mutants of Upland Rice Variety Nagina22: Generation and Characterization. *Proceedings of the Indian National Science Academy*. 80(1):163-172. doi: 10.16943/ptinsa/2014/v80i1/55094.
- Oard JH, Linscombe SD, Braverman MP, Jodari F, Blouin DC, Leech M, Kohli A, Vain P, Cooley JC, Christou P (1996) Development, field evaluation, and agronomic performance of transgenic herbicide resistant rice. *Molecular Breeding*. 2(4):359-368. <https://doi.org/10.1007/BF00437914>.

- Quirasco M, Schoel B, Chhalliyil P, Fagan J, Galvez A (2008) Real-time and conventional PCR detection of Liberty Link rice varieties and transgenic soy in rice sampled in the Mexican and American retail markets. *Analytical and Bioanalytical Chemistry*. 392: 395-404.
- Rao AN, Jhonson DE, Sivaprasad V, Ladha JK and Mortimer AM (2007) Weed management in direct seeded rice. *Advances in Agronomy*. 93:153-155.
- Rao AN, Wani SP, Ramesha M, Ladha JK (2015) Weeds and weed management of rice in Karnataka state, India. *Weed Technology*. 29(1):1-7.
- Ruzmi R, Ahmad-Hamdani M, Abidin M and Roma-Burgos N (2021) Evolution of imidazolinone-resistant weedy rice in Malaysia: the current status, *Weed Science*. 1-11.
- Shoba D, Raveendran M, Manonmani S, Utharasu S, Dhivyapriya D, Subhasini G, Ramchandrar S, Valarmathi R, Grover N, Krishnan SG, Singh AK, Jayaswal P, Kale P, Ramkumar MK, Mithra SVA, Mohapatra T, Singh K, Singh NK, Sarla N, Sheshshayee MS, Kar MK, Robin S, Sharma RP (2017) Development and Genetic Characterization of a Novel Herbicide (Imazethapyr) Tolerant Mutant in Rice (*Oryza sativa* L.). *Rice*. 10(1):10. doi: 10.1186/s12284-017-0151-8.
- Shimatani Z, Fujikura U, Ishii H, Terada R, Nishida K, Kondo A (2018) Herbicide tolerance-assisted multiplex targeted nucleotide substitution in rice. *Journal Data in Brief*. 20: 1325-1331.
- Singh BK, Shaner DL (1995) Biosynthesis of Branched Chain Amino Acids: From Test Tube to Field. *Plant Cell*. 7(7):935-944. doi: 10.1105/tpc.7.7.935.
- Singh Y, Singh G, Johnson D, Mortimer M (2005) Changing from transplanted rice to direct seeding in the rice-wheat cropping system in India. K. Toriyama, K.L. Heong, B. Hardy (Eds.), *Rice Is Life: Scientific Perspectives for the 21st Century*, International Rice Research Institute, and Tsukuba (Japan): Japan International Research Center for Agricultural Sciences, Los Baños (Philippines). pp. 198-201.
- Singh Y, Singh VP, Chauhan B, Orr A, Mortimer AM, Johnson DE, Hardy B (2008) (Eds.), *Direct Seeding of Rice and Weed Management in the Irrigated Rice–Wheat Cropping System of the Indo–Gangetic Plains*, International Rice Research Institute, Los Baños, Philippines: Directorate of Experiment Station, G.B. Pant University of Agriculture and Technology, Pantnagar. pp. 191-203.
- Smith Jr RJ (1983) Weeds of major economic importance in rice and yield losses due to weed competition. *Proceeding of the conference on weed control in Rice*. 31: 19-36.

- Sun Y, Zhang X, Wu C, He Y, Ma Y, Hou H, Guo X, Du W, Zhao Y, Xia L (2016) Engineering Herbicide-Resistant Rice Plants through CRISPR/Cas9-Mediated Homologous Recombination of Acetolactate Synthase. *Molecular Plant*. 9(4):628-631.
- Tian X, Hao J, Fang B, Geng PP, La HG, Huang DN, Wang HQ (2015) Transformation of upland rice with the *bar* gene and selection for resistance to the herbicide Basta. *Euphytica*. **205**: 151–167. <https://doi.org/10.1007/s10681-015-1416-1>.
- Xiao G, Yuan L and Sun SSM (2007) Strategy and utilization of a herbicide resistance gene in two-line hybrid rice. *Molecular Breeding*: **20**: 287-292. <https://doi.org/10.1007/s11032-007-9091-0>.
- Xiao G (2009) Recent Advances in Development of Herbicide Resistant Transgenic Hybrid Rice in China. *Rice Science*. 16(3):235-239.
- Yaduraju NT, Mishra JS (2008) Sedges in rice culture and their management. In *Direct Seeding of Rice and Weed Management in the Irrigated Rice-Wheat Cropping System of the Indo-Gangetic Plains*; Singh Y, Singh G, Singh VP, Singh P, Hardy B, Johnson DE, Mortimer M, Eds.; Directorate of Experiment Station, G.B. Pant University of Agriculture and Technology: Pantnagar, India; pp. 17.
- Yi SY, Cui Y, Zhao Y, Liu ZD, Lin YJ, Zhou F (2016) A novel naturally occurring class I 5-enolpyruvylshikimate-3-phosphate synthase from *Janibacter* sp. confers high glyphosate tolerance to rice. *Science Report*. 619104.
- Zhao T, Lin C, Shen Z (2011) Development of Transgenic Glyphosate-Resistant Rice with G6 Gene Encoding 5-Enolpyruvylshikimate-3-Phosphate Synthase. *Agricultural Sciences in China*. 10(9): 1307-1312.
- Zhang R, Chen S, Meng XB, Chai ZZ, Wang DL, Yuan YG, Chen KL, Jiang LJ, Li JY, Gao CX (2020) Generating broad-spectrum tolerance to ALS-inhibiting herbicides in rice by base editing. *Science China Life Science*. 64:1624-1633.





ICAR-National Rice Research Institute

Cuttack - 753006, Odisha, India

Phone: 0671-2367757, Fax: 0671-2367663

Email: director.nrri@icar.gov.in

directorcrricuttack@gmail.com

Website: <http://www.icar-nrri.in>



(An ISO 9001 : 2015 Certified Institute)

