ADVANCES IN
RICE BREEDING
Stress Tolerance, Climate Resilience, Quality & High Yield

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Advances in Rice Breeding :

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FOREWORD

Rice is the most widely consumed food for about half of the global population. Globally, the crop is cultivated in about 160 million hectares with production of approximately 500 million tons of milled rice. India has the largest rice area but the growing environments are characterized by widely diverse and highly challenging. Rice plays a pivotal role in the national food and nutritional security as it is the staple food for more than 65% of Indian population which contribute to about 40% to the total food grain production of the country. The increase of 2-3 million tons of additional milled rice per year must be achieved from less land, water, labour and chemicals under the adverse effects of the climate change.

Continued efforts and the approaches followed for rice improvement have significantly progressed in breeding techniques. The challenges of rice production under the climate change are being addressed using the modern tools and techniques. Simultaneous incorporation of multiple stress resistance/tolerance genes/QTLs will be crucial for developing climate-smart rice varieties. Utilization of high throughput genotyping, phenotyping and genomics-assisted breeding will be increased to assemble/pyramid desirable genes for stress tolerance, quality improvement and yield enhancement. Use of cutting-edge technologies provides opportunity to break the yield ceiling even in new generation climate-ready rice varieties.

Concerted research efforts of rice scientists and support from Government agencies have enabled the farmers to produce >117 million tons of milled rice during 2019-20. The production achievement after meeting the domestic demand has allowed export of >15 mt rice. However, for ensuring the food and

nutritional requirement of the projected increasing population under the climate change adversities, research efforts for sustainable rice production need to be continued.

This book on "**Advances in rice breeding: stress tolerance, climate resilience, quality and high yield**" published by ICAR-National Rice Research Institute, Cuttack, Odisha reports the major breeding strategies and research advancements in rice improvement during past few decades. The chapters included in this book are rightly selected for systematic rice improvement needed for future programmes. Further, the emerging areas of research on development of climate-smart rice, enhancing productivity, nutrient dense rice, speed breeding and other areas using molecular techniques including genome editing have been focused in this book.

I congratulate the editors and authors of different chapters for their contributions in bringing out this useful publication that would serve as a reference book for researchers, students and policy makers. I wish, the book will help achieve targets by the stakeholders under the emerging scenarios.

(T Mohapatra)

PREFACE

Rice is life for millions of people in the globe. Importance of rice in India is easily visualized from its use as worship material for ceremonies and rituals. Rice is the staple food for more than half of the world population. It provides important compounds such as carbohydrates, quality proteins, vitamins, many minerals, dietary fiber, specific oils, and a few phyto-compounds that provide health benefits. It is the predominant dietary energy source and economically, socially and culturally considered important for consumers in many Asian countries, where 90% of total rice is produced and consumed. Rice availability in many Asian countries determines food and livelihood security and political stability.

In terms of area rice crop is grown in about 44.0 million hectares in India, which is about 35.16 per cent of net cropped area and 43.86 per cent of area under cereals, which is the largest acreage in the world. India is the second largest rice producing country in the world. Even within the country, rice is the major cereal crop which accounts for 40.5 percent of total food grain production and 43.42 percent of total cereal production thus continues to hold the pivotal role to sustain food sufficiency in the country. Rice crop is providing livelihood to about 4 billion people, which constitutes about 55% of the global population. The rice production recorded a steady upward trend during the last five decades with a threefold increase in production from 34.5 million tons in 1960-61 to 116.42 million tons in 2018-19. Thus the country has witnessed impressive production and productivity growth trends during eighties, although declined, sustained during nineties due to wide scale adoption of high yielding rice varieties released over past five decades. As a result of enhanced rice production the country made a mark in international trade by becoming the largest exporter of rice in the world earning considerable foreign exchange.

Being the staple for 65% of the population, at the current level of per capita availability rice production is required to go up by 40 and 70% - from 116 to 145 and 190 million tons of milled rice respectively by 2030 and 2050. We would be therefore, requiring to add annually not less than 3.0-3.5 million tons of milled rice to sustain the present level of self-sufficiency in rice. It is therefore, a challenging task to achieve this targeted production levels in the next few decades as increase in productivity has to come from the declining and degrading resource

base in terms of land, water and other inputs and demand for environmentally sound rice production practices.

Of the 44 million hectares of rice area, irrigated area constitute 55.9 percent, contributing 75.5 percent production with productivity level of 2.87 t/ha., about 31.9 percent are rainfed low lands contributing 26.7 percent production with productivity level of 1.5 t/ha. 9.24 percent rainfed uplands contribute 5.8 percent production with a productivity level of less than 1.0 t/ha and 2.96 per cent area is deep water with stagnant flooding contributing 4.0 percent production with a productivity level of 1.4 t/ha which profoundly influenced the overall productivity of the country and such vast areas under each ecosystem is not encountered in any other rice growing country. However, it is estimated to achieve production by 70 per cent from irrigated ecology, 5 per cent from uplands, 21 per cent from rainfed medium lands and shallow low lands and 4 per cent from flood prone systems. Rice varieties with higher yield potential must be developed to enhance the average farm yields of rice lands in the country.

Practically with no scope for horizontal growth, the projected demand of rice has to be largely achieved through vertical growth and progressive productivity/production growth of rice being crucial for sustaining self-sufficiency. During the last decades significantadvancement has been made in developing high yielding rice varieties tolerant to biotic and abiotic stresses through innovative breeding and selection techniques. The convergence of conventional and modern molecular techniques has made precise and accelerated improvement in rice research. Concerted research efforts are being made to tackle the problems and drawbacks in rice production, productivity, nutritional quality, and resilience to various biotic and abiotic stresses. However, the major challenges of climate change, water availability, soil health, nutrient utilization efficiency, increased emergence of insects and diseases should be addressed to break yield ceiling and develop rice varietiesfor enhanced productivity and higher profitability. New and emerging breeding strategies have been worked out by involving genomic selection and breeding approaches are beingaccelerated and adoptedto develop breeding products in minimum time with greater efficiency and precision.

Through the publication of this book on **"Advances in rice breeding: stress tolerance, climate resilience, quality and high yield"** by ICAR- National Rice Research Institute, Cuttack, Odisha an effort has been made to review the accomplishments in conventional rice breeding to genome editing, genomics and other approaches for rice improvement. The chapters cover advances in conventional rice breedingapproaches,utilization of genetic resources, systematic rice improvement in different rice ecosystems,disease and insect resistance, hybrid rice, grain quality traits, seed quality parameters and emerging areas of research

on climate-smart rice, nutrient dense rice, water and nutrient use efficiency, speed breeding, double haploids and genome editing.

During the preparation of the book the authors were extremely supportive and we express our sincere thanks and gratefulness to our esteemed authors. Without their sincere, determined and timely effort, the publication of this book would not have been possible. We hope that this book will serve as a comprehensive reference material for rice researchers, graduate students and teachers involved in rice improvement.

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CONTENT

Sl.No.	TOPIC	PAGE
1	Foreword	
2	Preface	
3	Conventional breeding approaches for meeting the future demands in rice: A critical analysis ..... 01 *SR Das, BCY Collard and SK Pradhan*	
4	Utilization of Genetic Resources including wild species for Rice Improvement ..... 31 *BC Patra, Anilkumar C and M Chakraborti*	
5	Rice improvement for upland ecology ..... 42 *NP Mandal and S Roy*	
6	Genetic Improvement of Rice for Lowlands ..... 63 *TP Azharudheen, RP Sah, D Moharana, S Behera and SK Pradhan[1]*	
7	Irrigated Rice Breeding ..... 86 *SK Dash, L Behera, RP Sah, RL Verma, Anil C, M Azaharuddeen, K Chakraborty*	
8	Challenges in rice improvement for the coastal ecosystems of India under changing climatic scenario ..... 128 *K Chattopadhyay, BC Marndi, K Chakraborty, LK Bose, A Poonam, KA Moll[1], Shridevi A Jakkeral, V Vighneswaran, KK Manohara, S Thirumeni, KR Rao and RK Sarkar*	
9	Climate-Smart Rice Breeding: Progress and Challenges for the Rain-fed ecologies in India ..... 144 *SK Pradhan, E Pandit, SR Barik, SP Mohanty, DK Nayak, RP Sah, L Behera, P Sanghamitra, LK Bose and SR Das*	
10	Hybrid rice research and imminent prospects ..... 163 *RL Verma, D Jena, D Rout, JL Katara, R Raj, S Sarkar, B Sahoo, A Singh, P Arsode, C Mohan, SD Mohapatra, AK Mukherjee, S Samantaray and BC Patra*	

Sl.No.	TOPIC	PAGE
11	Molecular breeding for diseases and insect pest resistance in rice *P Samal, A Bal, P Sahoo, M Behera, D Dalai, BC Patra, AK Mukherje, GP Pandi G, SR Prabhukarthikeyan, S.K Lenka, PC Rath, LK Bose, M Azaharudheen TP, L Behera, KA Molla, M Chakraborti and MKKar*	185
12	Genetic solutions to improve nutrient use efficiency in rice *A Anandan, S Panda and J Meher*	232
13	Molecular breeding for genetic improvement of grain quality in rice *S Sarkar, P Sanghamitra, N Basak, G Kumar, R Reshmi, T Adak, TB Bagchi, K Chattopadhay, M Chakraborti, A Anandan, HN Subudhi, J Meher, SK Pradhan and SR Das*	250
14	Genetic enhancement of seed quality traits in rice *P Sanghamitra, R Bastia, J Mishra, S Sahoo, SK Pradhan*	280
15	Speed breeding - A unique tool for higher genetic gain in rice *RP Sah, Anil Kumar, SK Dash, Md Azharudheen TP and SK Pradhan*	316
16	Doubled Haploid Technologies for Rice Improvement: Technical Progress and Prospects *S Samantaray, B Dash, SS Bhuyan, Devanna BN, Parameswaran C, JL Katara, RL Verma and P Rout*	328
17	Genome editing for rice improvement *S Karmakar, T Meenkashi, MJ Baig, Parameswaran C and KA Molla*	352
18	Genomics and other omics approaches for rice improvement *L Behera, S Sekhar, S Mohanty, Devanna BN, C Parameswaran and SK Pradhan*	369

Conventional breeding approaches for meeting the future demands in rice: A critical analysis

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SUMMARY

By the adoption of semi dwarf rice varieties, rice growing nations have made spectacular advance in rice production during last few decades, enabling to a period of self-sufficiency and surplus. However, national and international rice improvement programs have not made any significant increase in the genetic yield potential of varieties since the release of IR8 and have only added values to the semi dwarf high yielding varieties by insulating them with resistance / tolerance to biotic and abiotic stresses. If this trend continues in coming years too then it may not be possible to sustain the self-sufficiency in rice. Therefore, there is a need to raise the genetic ceiling to yield by integrating both conventional and molecular approaches in rice. The conventional research strategies namely use of efficient mating systems to enhance genetic variability, exploiting the gene pool concept and use of weedy relatives, use of disruptive mating and innovative breeding and selection approaches, male sterile facilitated composite, recurrent selection and population improvement, scope for the development of heterogeneous population and breeding methodology for converging conventional with molecular breeding are suggested to enhance the genetic yield potential of semi dwarf high yielding varieties in rice.

Keywords: Gene pool, recurrent selection, population improvement, heterogeneous population

1. INTRODUCTION

Rice is the major food crop of the world and is the staple food for more than 60 percent of global population in providing their calorie needs and nutritional requirement. It is the predominant dietary energy source and economically, socially and culturally considered important for consumers in many Asian countries, where 90% of total rice is produced and consumed (Siddiq and Vemireddy, 2021). In majority of the rice dependent developing economies, rice availability determines food and livelihood security and political stability (Rice Almanac, 2013).

The rice production in India recorded a steady upward trend during the last five decades with almost four fold increase in production from 34.5 million

tonnes in 1960-61 to 121.46 million tonnes in 2020-21. This has helped in bringing down the quantum of imports and helped in achieving self-sufficiency in food production by mid 1980s. Thus the country has witnessed impressive production and productivity growth trends during eighties, although declined, sustained during nineties due to wide scale adoption of high yielding rice varieties released over past five decades. As a result of enhanced rice production, the country made a mark in international trade by becoming the largest exporter of rice in the world earning considerable foreign exchange of about Rs. 53,000 crores during 2018-19.

Being the staple for 65% of the population, at the current level of per capita availability rice production is required to go up by 40% and 70% from 116 to 145 and 190 million tonnes of milled rice respectively by 2030 and 2050. We would be therefore, requiring to add annually not less than 3.0-3.5 million tonnes of milled rice to sustain the present level of self-sufficiency and surplus in rice. It is therefore, a challenging task to achieve such a huge targeted production levels in the next few decades as increase in productivity has to come from the declining and degrading natural resource bases like arable land, irrigation water, inputs, genetic resources and demand for environmentally sound rice production practices. Practically with no scope for horizontal growth, the projected demand of rice has to be largely achieved through vertical growth. Progressive productivity/production growth of rice being crucial for sustaining self-sufficiency and marching towards food security, planning and prioritizing rationally research and development strategies are important for achieving the near- and long-term physical availability (Siddiq, 2013; Babu et al., 2015).

It is a disquietening feature to note that the impressive production and productivity growth rates during eighties has come down during nineties both in India as well as in Asia (Table1). However, the abysmal growth rate trends during 2000-2020 indicate that there is instability in rice production and which has been a matter of concern in India. The possible reasons for instability were due to changes in cropping pattern, insignificant increase of irrigated area to total cropped area, decline in use of improved seeds and manure and other inputs necessary for agriculture (Jain, 2018; Senapati and Goyari, 2019). Further, the experimental evidences have clearly shown that important rice improvement programs in India as well and at the International Rice Research Institute, have not made any significant increase in the genetic yield potential of varieties released after IR8 and no perceptible yield improvement has been achieved since 1980 (Virmani et al., 1993; Khush, 1995; Peng et al., 1999). If this trend continues in the coming years too and if we fail to stabilize the yield growth level at least at where we stand today, then it may not be possible to sustain the level of sufficiency the country has achieved in rice. Therefore, there is a need to make concerted

Table 1. Growth rates (%/year) of rice area, production and yield in India and Asia

Region	Period	Area	Production	Yield
India	70-79	0.87	1.88	1.01
	80-89	0.41	3.58	3.17
	90-99	0.67	2.00	1.33
	00-09	-0.02	1.62	1.64
	10-19	0.15	1.82	1.67
Asia	70-79	0.73	2.33	1.60
	80-89	0.18	2.54	2.36
	90-99	0.64	1.70	1.07
	00-09	0.50	1.81	1.31
	10-19	-0.20	0.74	0.94

research efforts to raise the genetic ceiling to yield only through vertical yield growth.

Plant breeders today argue that, there is hardly any evidence of yield plateau for any crop including rice. Siddiq (1991), has pointed out that continuous growth, no doubt with plateaus at different phases since 1900 suggest that, a perfect variety has yet not been evolved. Still there is an unexploited genetic variability for improving the direct and indirect components of yield through utilization of untapped exotic germplasm. There is enough scope to develop and use more efficient breeding and selection techniques for yield improvement in rice.

2. MATING SYSTEMS

Biparental crosses are widely used in rice varietal improvement programs to serve the purpose of combining simply inherited traits from two parents. Since the introduction of nitrogen responsive high yielding dwarf varieties, the breeding, objectives have become more and more diverse and it was no more possible to combine desired characters to desired norm through biparental crosses or in other words the recombination possible from biparental crosses are too restrictive to make rapid improvement in a selfing species like rice.

To overcome this genetic limitation, the use of multiple crosses as suggested by Harlan, Martini and Stevens (1944) involving 16-32 parents which are crossed in successive generations until the final hybrid involve all the parents, could be effectively used in rice. Theoretically the multiple crosses provide an opportunity for recombination of genes from many parental strains by intermating F_1s in successive generations. However, it is practically not possible to get enough F_1

seed in later generations of mating cycles to retain all the parental genes in the final hybrid. The second practical limitation was suggested by Mackey (1954) that by using 16-32 parents in a multiple crossing program will force the inclusion of number of unadapted strains, which are likely to disrupt the good genetic background of varieties that took several years even decades to assemble and therefore suggested modified backcrossing programs to obtain optimum parental lines and each unadapted parent should be crossed and backcrossed to an adapted variety before its use in a multiple crossing programs (Fig. 1).

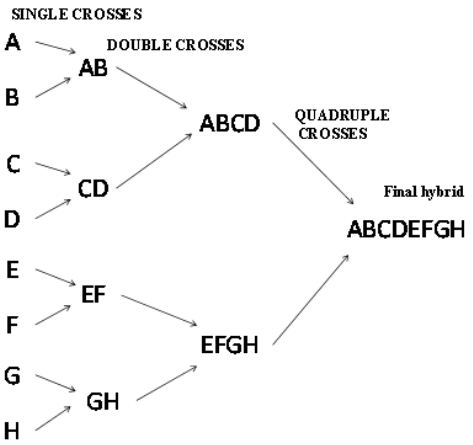

Fig.1. Multiple Crossing Program

Three-way crosses were tried in many selfing species where the third parent is another adaptable germplasm, serves the purpose of increasing the proportion of adaptable germplasm and simultaneously widen the scope for combining desirable genes from another good parent. It has been observed that the populations of three-way crosses had larger genetic variance than biparental crosses and produced more superior lines than two-way crosses. Such crosses also provide an element of gamete selection, since the superior performance of test cross F_1 plant reflects the combination of superior gametes from the segregating single cross with genotypes of the non-segregating variety as the third parent. As argued by Stadler (1944) the frequency of superior gamete is much higher than the frequency of superior zygote and hence three-way crosses provide a more efficient mating system for obtaining lines with better performance (Sharma, 1989). Double crosses involving four different parents were also useful in extending the range of parents and achieve recombination of desirable alleles from diverse parents.

The different mating systems as suggested were limited to a great deal by the requirement of large number of F_1 seeds. As the F_1 generation serves itself as the segregating population similar to F_2 of single crosses and set the limit of possible recombination of genes available in the population. Never the less, these multiple crosses are expected to generate an array of genotypic variability which never existed earlier in any breeding population.

Multiple crossing has hardly been employed in rice breeding programs. It is interesting to note that out of 427 rice varieties released up to 1992 in India (37 in sixties, 153 in seventies, 205 in eighties and 32 varieties in nineties) only one variety was derived from multi-parents, thirteen from three-way crosses and three

varieties were double cross progenies. It is not surprising that many rice breeders have not used multiple crossing programs for varietal improvement in rice because they could get adequate success through careful manipulation of biparental progenies. However, the limited success achieved through various mating schemes employed in rice is primarily ascribed not only to the requirement of large number of F_1 seeds but also relatively small sample size in F_2, where the full range of yield is not realized. Still there is scope to make use of multiple crossing programs for creation of a wide array of genetic variability and which would facilitate selection of superior individuals for substantial yield improvement in rice. A majority of breeders have restricted their selection to known material and have made intense efforts for local adaptation, as a result certain gene blocks are rapidly fixed along with correlated response which in many cases are adverse in direction. Therefore, it is suggested to choose the parents on the basis of wide genetic base with acceptable level of productivity and high general combining ability before their use in a multiple crossing program for realization of high and stable yields in rice.

3. GENE POOL CONCEPT AND USE OF WEEDY RELATIVES

The gene pool concept for plant breeding was conceived by Harlan and de wet (1971) which divides the total array of genetic variation within the reach of plant breeders, into primary, secondary and tertiary gene pools. Often the primary gene pools include the progenitor of cultivated species that can easily be crossed with cultivated type and produce fertile hybrids with normal segregation.

Frey and his group over a period of 20 years have successfully demonstrated that the ancestral forms *Avena sterilis* in oat occur in the primary gene pool, has been a valuable source of germplasm for increasing crop productivity. The interspecific oat matings between. *A. sativa* and *A. sterilis* showed a great deal of transgressive segregation and Frey (1975) have shown that a considerable amount of yield improvement ranging from 3 to 29 per cent was achieved through such matings, while the agronomic traits similar to the recurrent parent was maintained in BC_3 to BC_5 generations. Further, three elite lines were derived from the second cycle of breeding with *A. sterilis*, showed a considerable yield improvement of 26 to 31 per cent and were subsequently released to Midwestern USA farmers. The development of elite lines from such matings takes advantage of nuclear genes from *A. sterilis* and thus adds a quantum increase in yield potential of cultivated oat varieties in USA.

Similarly, results from introgression of the germplasm from *H. spontaneum* into cultivated barley to increase productivity are equally as exciting as those from oat study (Rodgers, 1982). Promising barley lines with impressive yield advance (29 to 46 %) and with moderate to good levels of agronomic traits were realized in BC_3 to BC_5 generations. In those back cross generations, the expected

proportion of germplasm from wild species is 6.25 % or less and it is a general phenomenon that both oat and barley can tolerate about the same percentage of wild germplasm for expression of improved productivity.

Nayar (1973) reviewed the genomic relationship among *Oryza* species and established that both the cultivated species *O. sativa* and *O. glaberrima* and their related wild species such as *O. nivara, O. rufipagon, O. breviligulata* and *O. longistaminata* have the same AA genome and they share a common gene pool. These weedy species can be easily crossed with cultivated types and their F_1 hybrids have normal chromosome pairing but show varying levels of sterility. More often the wild species in rice have been used for transferring resistant genes stress tolerant genes into cultivated type but never been systematically exploited for increasing higher productivity in rice. Therefore, it is suggested to make use of weedy and wild species from the primary gene pool and examine the feasibility of wild germplasm for improving yield potential, like oat and barley, in rice.

4. DISRUPTIVE MATINGS IN PLANT BREEDING

When breeders attempt to make use of unthrifty wild or weedy sources for yield enhancement the chances of success are remote due to the problems of linkage. Linkages drag undesirable alleles into the breeding population as is usually linked to favourable gene and thus discourage breeders from using exotic germplasm sources. There are several instances to demonstrate that repulsion phase of linkage does not express a portion of the potential genetic variation for a trait. To overcome this undesirable genetic limitation Mather (1953, 1955), Thoday (1958, 1960) had suggested the use of disruptive mating in cereals (Fig. 2).

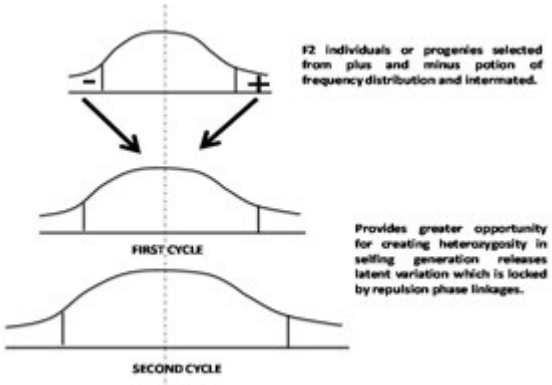

Fig. 2 Disruptive Matings in Plant Breeding

Generally, samples of individuals or progenies are selected from plus and minus portions of the frequency distribution and only plus and minus matings are made. Over cycle of such disruptive matings, it is seen that the population mean remain unchanged but the variance and range of the frequency distribution is increased many folds (Blumer, 1976). Theoretically disruptive mating via mating unlike offers greater opportunity for creating heterozygosity in selfing generations and enhances rapid crossing over and releases latent variations which are locked up by repulsion phase of linkages. Although, through such matings the mean

remained unchanged but it does not necessarily mean that superior individuals with improved yield potential and better agronomic features could not be recovered. The value of disruptive mating for improvement of flowering period, plant height and grain yield in *Brassica campestris* var. *brown sarson* has been experimentally verified by Murty et al. (1972) which confirmed the breaking up of repulsion linkages for increasing the genetic flexibility of the population. However, Frey (1984) is of the view that disruptive mating is considered to be an effective plant breeding procedure as more exotic germplasm sources would be used in future plant breeding programs for improving the direct and indirect components of yield.

5. INNOVATIVE BREEDING AND SELECTION APPROACHES

So far not many rice breeders have utilized novel breeding or selection approaches in the tropics as the pedigree selection in biparental progenies having provided them adequate success (Ikehasi and Fujimaki, 1980). However, when the breeding objectives become more and more diverse and even when complex crosses are made for combining desired traits from different parents the period of fixation time is enhanced. More complex the trait is genetically longer period it takes to get fixed. Therefore, the rice breeders were compelled to make use of more efficient breeding/selection methodologies to overcome various genetic limitations for rapid progress in varietal improvement in rice.

Single seed descent (SSD) a modification of original bulk method, is a breeding procedure in which fixation of traits with minimum bias from potential variability is achieved. To prevent any drift in segregating populations, a single seed should be harvested from each F_2 plant to grow F_3 population and that the same procedure is repeated until F_3-F_6. This method is applicable to self-pollinated field crops for which growth conditions can be manipulated to enforce earlier flowering and seed set compared to normal field conditions. Typically, plants are grown in a greenhouse or screenhouse facility and several generations or cycles are completed between F2 and F6 generations within a quicker time compared to normal field conditions. Therefore, this method has also been referred to as rapid generation advance (RGA). In the literature, SSD and RGA are typically used interchangeably (Collard et al., 2017).

Rapid generation advance (RGA) is not a breeding method but a way of growing bulk populations. Since the major objective of SSD is to attain homozygosity prior to pedigree selection, it becomes essential for rapid cycling of breeding materials that shorten the growth duration of a given population through short day treatment. A large number of plants can be accommodated in limited space and dense spacing as only one or two seeds from each plant to make a composite population which reduces the genetic drift and is used to advance the

segregating generations. Thus, the SSD method is most appropriate for RGA and which is used as a routine breeding method in order to improve breeding efficiency (Collard et al., 2017). Success in RGA largely depends on minimum growth duration that can be induced in a specific growth condition. It has been demonstrated that for breeding photoperiod sensitive rice in the tropics, three generations a year can be grown through RGA (Ikehasi and Nieto, 1977; Ikehasi and Hille Ris Lambers, 1979). It has been suggested that there would be higher genetic gain by employing SSD method of breeding coupled with RGA simply by modifying the empirical formula used in selection theory. The formula for genetic gain (ÄG, or response to selection), is given by: $ÄG = (ih^2 ó_p)/ L$ where, i is the selection intensity, h^2 is the trait heritability, $ó_p$ is the square root of the phenotypic variance and L is the length of the breeding cycle or generation interval. Genetic gain is increased due to reducing of the time of breeding cycle (Atlin et al., 2017; Collard et al., 2017, and Collard et al., 2019).

Use of RGA in rice breeding: Bulk method of breeding or its modified versions like single seed descent (SSD) method coupled with rapid generation advance (RGA) was successfully employed for rice breeding in Japan and steadily gained popularity among rice breeders (Kikuchi, 1978). The major objective of SSD method is to attain homozygosity earlier to pedigree method of selection when it is coupled with RGA. It has been effectively utilized for breeding photosensitive long season varieties (Ikehasi and Nieto, 1977). Twenty-four leading Japanese varieties which covered over 40% of the total rice area were bred using RGA by 1977 (Ikehasi and Fujimaki, 1980). This method could be used to shorten the breeding cycle and was applied for cold tolerance breeding (Heu et al., 1982). The use of RGA in Japan led to the development of the extremely popular rice variety called Nipponbare, which was used to generate the first rice genome sequence (Collard et al., 2017). RGA was popularized at IRRI during the 1980s, especially for rain-fed rice and even deep-water rice. One advantage of using SSD was to manage photoperiod sensitivity present in many parents used for breeding for rain-fed ecosystems (Ikehashi, 1977), because some parents would not flower in field conditions.

Vergara et al. (1982) proposed that screening for highly heritable agronomic traits like earliness and plant height, tolerance to bacterial leaf blight and submergence tolerance could also be performed during RGA. RGA method was first reported in 1939, (Goulden, 1939) but it was not popular until the 1960s and 1970s after its use in barley, soybean and oats. In recent years, this method has gained more wide-spread use in molecular genetics research to develop mapping populations called recombinant inbred lines (RILs) for quantitative trait loci (QTL) mapping. These populations are ideal for QTL mapping because they are genetically homozygous and seed can be multiplied in large amounts

permitting phenotyping for many traits over many years (Collard et al., 2005; Collard et al., 2017).

One of the major obstacles of breeding long duration photosensitive varieties for integrating tolerance to submergence and stagnant flooding is the time required for fixing genotypes with desired character combinations. Several techniques have been developed to reduce the breeding and selection cycles in rice breeding. Single seed descent method (SSD) coupled with Rapid generation advance (RGA) is well suited to breeding long duration photosensitive varieties under field condition. This approach has been effectively and widely used by the NARS and IRRI. Ikehasi and HilleRislambers (1977, 1979) discussed the details of this method in relation to rice breeding and also suggested for an international integrated breeding programme for the development of photoperiod-sensitive rice (Fig. 3).

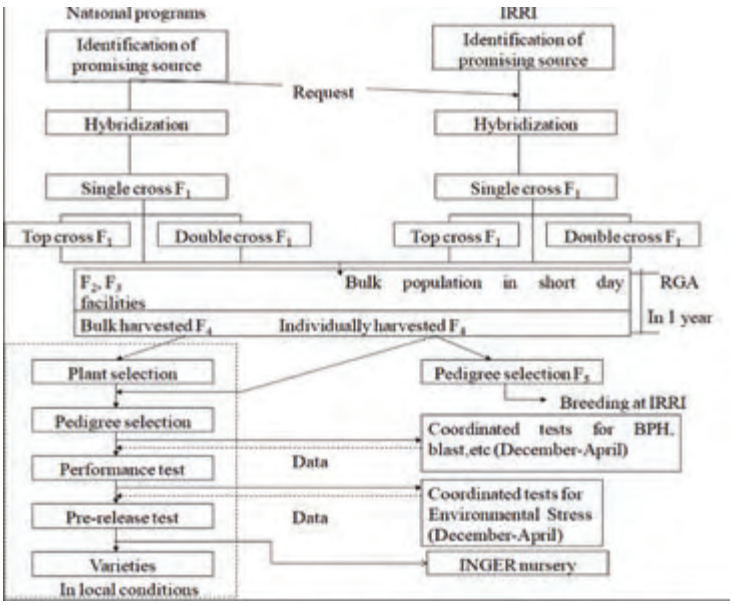

Fig. 3 Integrated international breeding program for photoperiod sensitive rice (Ikehachi and HilleRis Lambers,

6. COMPARISON OF PEDIGREE, SSD AND BULK METHOD OF BREEDING

Pedigree and mass pedigree including SSD method of breeding have their unique advantage and preference. But their relative efficiency in terms of recovery of frequency of desirable genotypes is yet to be ascertained. Casal and Tigehelaar, (1975) compared pedigree selection (PS), single seed descent method (SSD) and bulk population methods (BP) through computer simulation studies. They found that for characters with high heritability, PS was most effective while SSD was found to be most effective for traits with low to very low heritability (even upto 10%) and also offered the greatest benefits in situations where simultaneous selection for several traits having different heritability has to be done.

Knot and Kumar (1975) and Fahim et al. (1998) from two crosses in wheat and rice respectively compared F_6 lines derived through PS and SSD in replicated yield trials and established that the mean yield of PS lines was significantly higher

than those of SSD lines. A comparison of 20% highest yielding lines derived from both the methods indicated that the SSD lines were at least as good as PS lines. Studies by Pawar (1985) clearly indicated that SSD method was found better than bulk method of breeding and PS was superior for grain yield/plant and other yield components than that of BP and SSD populations.

It is specifically mentioned that only three published research studies have compared pedigree, RGA and other breeding methods in rice (Mishra et al., 1994; Fahim et al., 1998; Kanbar et al., 2011). Mishra et al. (1994) reported that bulk and SSD were superior in terms of generating higher yielding lines compared to the pedigree method. Kanbar et al. (2011), compared pedigree, modified bulk and SSD methods using F_3, F_4 and F_5 and F_6 populations derived from a single cross (Moroberekan/IR20). Twenty families produced by each breeding method was tested in a randomized complete block design trial with four replications and observed for eight traits. The results indicated that the pedigree method was superior to SSD for grain yield (Kanbar et al., 2011). However, it should be noted that the basis of the conclusion was comparison of population means of the selected lines and multiple comparison tests were not performed, which was also the case for Mishra et al. (1994). It is more appropriate to compare the best lines produced from each of the different methods together, rather than specifically compare population means, because one of the ultimate goals of breeding is to identify transgressive segregants (Collard et al., 2017). The landmark study by Fahim et al. (1998) empirically compared different breeding methods in great detail.

The authors compared the performance of F_6 breeding lines derived through pedigree, bulk, modified pedigree and SSD using two breeding populations. F_6 selected lines were directly compared from each of the four breeding methods in a field trial. The initial population sizes used varied depending on the breeding method. However, the population size was relatively large. A total of 12 quantitative traits were examined including grain yield. It was interesting to note that none of the best lines produced by pedigree, bulk or modified pedigree were significantly better for any trait from either cross than the lines produced using SSD. In fact, some of the best lines for grain yield were derived from the SSD method (Fahim et al., 1998).

Quite often it is argued and apprehended those poor lines are carried forward during the line fixation process in RGA. Considering the cost of line fixation associated with RGA it was concluded that SSD was as effective as the other methods, less costly and more rapid (Fahim et al., 1998). Further, Janwan et al. (2013) used SSD method to develop 271 RILs and investigated 11 quantitative traits. Three lines were significantly higher yielding than the best check variety.

These results provide further independent verification that RGA is an effective breeding method that should be more widely used. Besides reducing the breeding cycle, it is considered to exhibit potential genetic gain (Pandey and Rajatasereekul, 1999).

Anther culture derived dihaploidy (DH) is another method of realizing homozygous recombinants from F_1 hybrid. It has potential use in self-pollinated crops like rice due to the advantage of recovering homozygous lines in the very next generation. There is enhanced selection response due to presence of additive genetic variance alone in the dihaploid population. This approach has been refined in rice. Besides shortening the breeding cycle, there is high probability of recovering desired genotypes in very small population.

This approach has been used at IRRI in breeding for tolerance to submergence, salinity and cold in collaboration with NARS. In spite of such unique advantage, this strategy is not of wide use due to very low frequency of regeneration especially in *indica* rice. Courtois, (1993) compared both SSD and DH methods for three populations and concluded that both the methods were equally effective. However, based on technical simplicity and cost, RGA has advantages over doubled haploids and could be successfully adopted by NARS in South and SE Asia.

Several workers have compared various breeding methods in predominantly self-pollinated crop plants. The published reports by Tee and Qualset (1975), Boerma and Cooper (1975), Haddad and Muehlbauer (1981), Pawar et al. (1986), Nanda (1990), Mishra (1991) and Mishra et al. (1994) reveal conflicting results on the superiority of one method over the other. Although a definite trend of efficiency of different breeding methods is suggested, there are several instances to show that the superiority of any breeding method in retaining the high yielding lines largely depend on the choice of parents in different crosses, genetic variance for different traits, sample size, selection accuracy during the segregating generations, genotype and environment interaction, genetic drift and changes in gene frequencies due to inbreeding and heritability of traits. As far as the knowledge goes there are very few reports in rice to establish a general trend of relationship and therefore, it is suggested to workout precise experiments to study the efficiency and effectiveness of different breeding methods, for realization of high yield in rice.

7. MALE STERILE FACILITATED COMPOSITE

In the pedigree method of breeding the chances of genetic recombination is too restrictive because the selfing populations rapidly approach homozygosity. Another method for increasing genetic variation is the composite method of breeding which has been extensively applied in barley breeding by Suneson (1956).

In this method F_2 of several crosses are bulked into a composite population and subjected to natural selection in diverse environments. The strategy enhances genetic variability which is only confined to two parental strains of each cross, as there is no outcrossing among hybrid plants in the population. But introduction of male sterility into the segregating population help to enhance recombination through natural crossing and Suneson showed that natural selection had caused the bulk population to become higher yielding in later generations. Several composites developed by means of the male sterility factors were investigated by Jain and Suneson (1966) for quantitative genetic changes in variability and productivity. These composites exhibited a very high degree of genetic variability and the increase of genetic variability however not accompanies correspondingly increased productivity.

Before using bulk population of breeding as an efficient tool for crop improvement, one must critically analyze the advantages and disadvantages of this method of breeding. Spitters (1979) argue that in bulk propagation, progress made in the later generation may be too slow to be of value to a breeder as the final yield level does not guarantee recovery of high frequency of high yielding lines. However, the bulk population breeding has its own merits under certain conditions. When an economically important trait for instance is controlled by several recessive genes, the chances of recovery of recessive types in later generations are more, which was pointed out as an advantage of SSD approach (Brim, 1966). Also, due to close linkage there is delay in fixation of characters and therefore, selection is advocated in later generation. Ikehasi (1977) has also shown that the proportion of good recombinants from undesirable linkages can be increased by F_4 and F_5 through additional recombination. The plant breeding theory suggests that delaying selection after several cycles of inbreeding may have advantages. The response to selection using fixed lines will be many times larger compared to a F_2 population because genetic variation is completely partitioned between lines and selection accuracy (*i.e.*, the ability to correctly identify superior lines that can be effectively commercialized) is greatly enhanced. Furthermore, selection of families for highly heritable traits is enhanced as well because family means are not confounded by within-line non-additive genetic variation (Collard et al., 2017). In fact, Kearsey and Pooni (1995) stated that 'unless the heritability is high, selecting in the F_2 generation gives very little return on effort' and 'inbreeding (*i.e.*, SSD) followed by selection on family means can improve selection response by up to a factor of five or more' (Kearsey & Pooni, 1995).

Finally, the most important advantage of bulk breeding is to handle large genotypic variation generated from multiple crosses or composite populations and the segregating population which cannot be managed through pedigree selection. Further the role of natural selection is most crucial for causing the

evolutionary changes in the mean expression of a trait in a bulk population of segregates in a selfing species and can exert strong selection pressure for characters of very high adaptation. Evidences suggest that natural selection would cause either no change or change in a desirable direction. Nevertheless, by artificial selection the bulk population may be shifted towards agriculturally desirable types. The original concept of the bulk population method has been remarkably diversified by SSD, RGA and male sterile-facilitated composites. Ikehasi and Fujimaki (1980) have pointed out that the breeders are now capable of integrating one or more of the bulk method-related means to build the most efficient system for any breeding objectives. It is particularly significant that modifications in bulk population are to handle maximum genetic diversity with least cost and time.

So far male sterility facilitated hybridization has not been employed systematically in rice breeding. Male sterile mutants have been developed from IR 36 at IRRI (Singh and Ikehasi, 1981). They have suggested an integrated method for combining pedigree as well as bulk methods to develop composite population in rice, through male sterile facilitated system, for higher recombination by breaking up parental linkage groups and which may help to form a better base population for the development of best yielding, highly homozygous and homogeneous lines (Fig. 4).

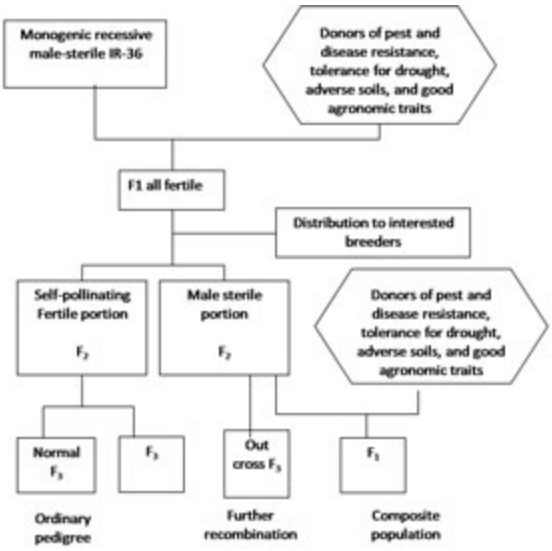

Fig. 4 Monogenic male sterility facilitated composite populations in rice

8. RECURRENT SELECTION AND POPULATION IMPROVEMENT

The plant populations developed from the introgression of exotic germplasm followed by disruptive mating could be subjected to any selection procedures immediately. For short term goals pedigree selection or some modifications would be appropriate while for long-term goals recurrent selection would be the suggested procedure (Frey, 1983, 1984).

Recurrent selection is primarily used to promote recombination and to increase the frequency of favorable genes for quantitatively inherited traits in population. It is cyclic and in each cycle two phases of plant breeding viz. selection of a group of genotypes that possess favourable genes and mating among the selected genotypes to obtain genetic recombination. Thus, it helps to gradually

increase the frequency of favourable genes until there is reasonable likelihood of obtaining the ultimate genotype in a finite sample. It can be applied to the improvement of quantitatively inherited traits in either allogamous or autogamous crop species, irrespective of the type of gene action involved in the determination of the trait. There are many instances to demonstrate that breeders have used this procedure mostly in cross-pollinated crop plants as there is no barrier in intermating favourable genotypes for enhancing genetic recombination among linked alleles in a population of plants.

But the obstacles to genetic recombination among alleles at closely linked loci still remained as a significant barrier to making improvement in self fertilizing species. With selfing heterozygosity which is necessary for effective crossing over and recombination of alleles at closely linked loci decreases very rapidly to an effective level. In order to avoid this genetic limitation Suneson (1945) introduced a male sterility gene into his famous barley composites to promote outcrossing and facilitate random mating without difficulty, for release of variability which is locked by linkage, which helps in the utilization of diverse sources, for practical crop improvement programmes. However, use of male sterility in the mating programmes has a decided selection disadvantage in the population and its frequencies decrease to a very low value within few generations and thus the effectiveness of this mechanism in promoting heterozygosis for increased crossing over would be ephemeral. There is also some evidence that too much crossing over in selfing species can be detrimental to fitness (Frey, 1975). Further if there is no prior selection of desirable types, the benefits of intermating among progenies as advocated by Hanson (1959) would not have any practical advantage over continued selfing. Therefore, particular attention should be paid to intermating in a system of recurrent selection.

Male sterile facilitated recurrent selection was first suggested by Gilmore (1964) for the improvement of predominantly self-pollinated crop plants. Application of genetic male sterility to recurrent selection was also proposed in breeding of sorghum and soybean (Doggett and Eberhart, 1968; Brim and Stuber, 1973).

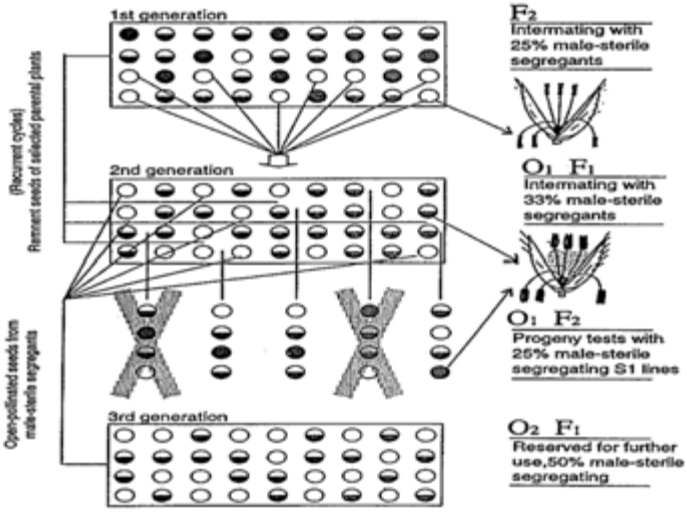

Fig. 5 Male-sterile facilitated recurrent selection for rice breed-

Male sterility facilitated recurrent selection schemes were systematically employed in rice breeding after the availability of a male sterile mutant of IR 36 at IRRI (Singh and Ikehasi, 1981). Fujimaki (1978, 1979) initiated programmes to transfer polygenically controlled horizontal resistance to blast and major-gene resistances to BLB into the most popular rice variety 'Nipponbare' in Japan through recurrent selections (Fig. 5). Similarly, it was also possible to obtain hybrid population closely resembling the recurrent parent 'Nipponbare' in days to heading, plant stature, grain shape and other morphological traits along with smoothness of rice plants which effectively reduce dust generated in harvesting, threshing and hulling operations through male sterile-facilitated backcrossing in rice.

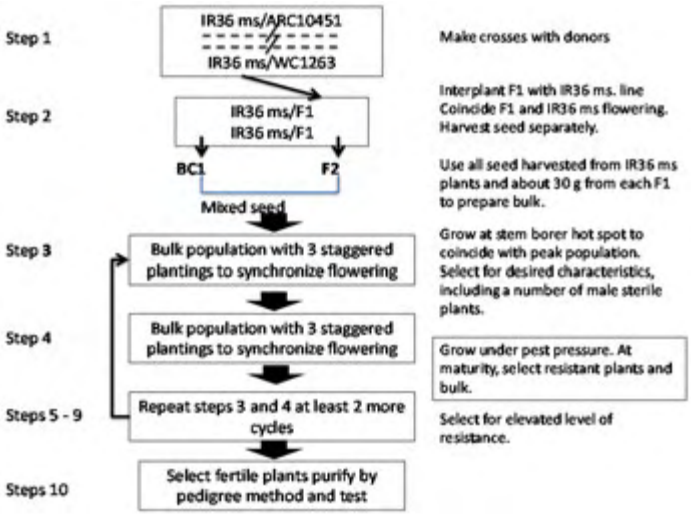

Fig. 6 Male-sterile facilitated recurrent selection to elevate level of Stem Borer resistance

Chaudhury et al. (1981) suggested a ten-step procedure, involving twenty-six stem borer tolerant donors, to increase the level of resistance to yellow stem borer through male sterile facilitated recurrent selection in rice (Fig. 6).

Sahai and Chaudhury (1993) also outlined a male sterile facilitated recurrent selection scheme for the development of rainfed lowland varieties in rice (Fig. 7).

Although many workers have outlined male sterile facilitated recurrent selections in rice, but an extensive recurrent selection project was initiated in

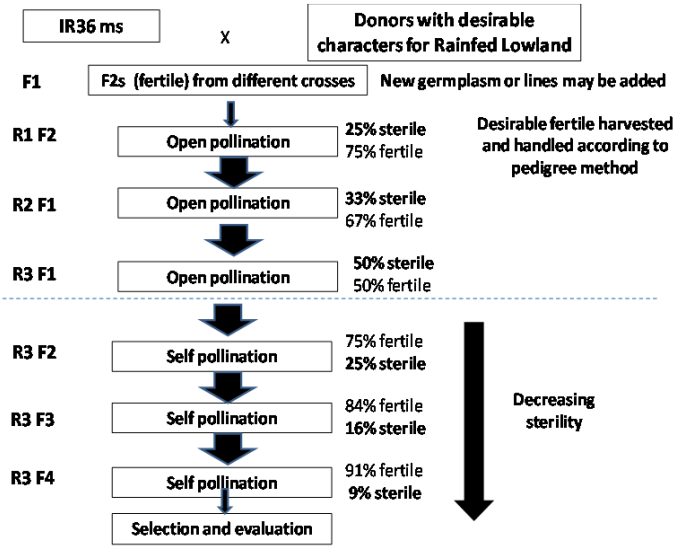

Fig. 7 Male-sterile facilitated recurrent selection in *rainfed lowland rice*

Brazil using the recessive male sterile gene of IR 36 in1984 under the auspices of CIRAD-CA, CIAT, WARDA and IRRI for broadening the genetic base of gene pools in tropical upland, japonica, lowland tropical indica, upland and irrigated tropical high altitude and irrigated temperate *japonica* rices. Several gene pools and populations were developed which have been used as genetic base population for recurrent selection in different Latin American countries like Brazil, Chile and Colombia and in several African countries viz. Ivory coast, Mal, and Madagascar. In 1989, the basic gene pools CAN IRAT-4 and 5 for tropical indica irrigated and tropical japonica upland conditions were made available to the international scientific community. In 1990, at CIAT a hand crossed recurrent selection project began focusing on the development of gene pools and populations targeting blast resistance in rice (Chatel and Guimaraes, 1994).

They have concluded that rice recurrent selection programme has been successful in reaching its maturity. It has derived new gene pools and populations targeting different rice ecosystems and improvement of specific traits like grain quality, resistance to blast, tolerance to drought and yield potential. CIRAD-CA and CNPAF developed the *indica* and *japonica* basic gene pools CAN-IRAT 4 and CAN-IRAT-5; CIRAD-CA and IRRI developed *indica-japonica* CPI 22L and indica CP 126 gene pools and CIAT developed the *indica-japonica* gene pool GC 91 by hand crossing.

Presently breeders have incorporated the recurrent selection method in their conventional breeding programmes, as a new tool to achieve diverse breeding objectives. Recurrent selection and traditional breeding have been used in a complementary way. This innovating breeding method has created a great deal of interest and anticipations among rice breeders for the development of rice varieties with increased yield, better resistance and greater stability.

9. POPULATION IMPROVEMENT

The success of recurrent selection has led to several population improvement schemes. Recurrent selection has been applied to closed genetic systems i.e., it was applied to genetically heterogeneous population of plants but no new germplasm was added to the population during the period when the procedure was being used. The potential limitations on progress from recurrent selection in closed populations have led to several proposals collectively known as 'population improvement' (Frey, 1984).

Eberhart et al. (1967) proposed a comprehensive breeding system for both allogamous and autogamous species. Jensen (1970) proposed the 'diallel selective mating' (DSM) system which was designed primarily for autogamous species. By this procedure the breeding populations of plants seen as a dynamic gene pool (a) to which new sources of germplasm are introgressed whenever feasible (b) in

which frequencies of favorable alleles are progressively increased via recurrent selection (c) in which genetic recombination is enhanced by massive hybridization among selected genotypes or in other words there is opportunity form multiparental gene recombination (d) from which cultivars, inbreds or parental lines can be extracted at any stage (Fig.8).

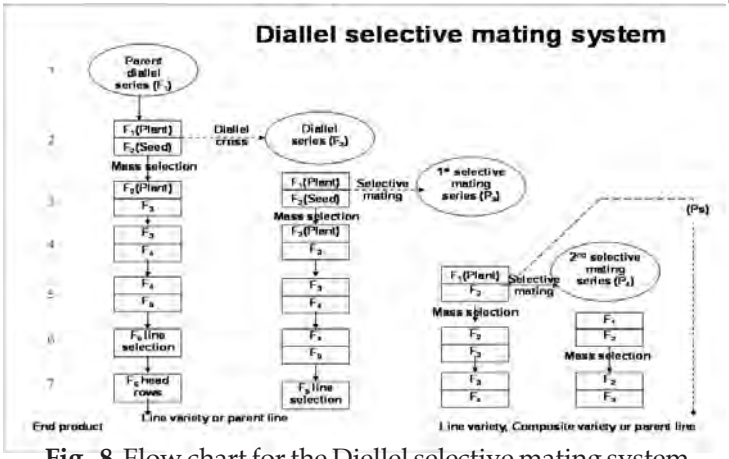

Fig. 8 Flow chart for the Diellel selective mating system (Jensen, 1978)

In diallel selective mating (DSM) the breeder selects the parental lines and crosses them in a diallel series (Column-1) and F_1s of the diallel series are used to produce F_2 seed for raising F_2-F_6 generations. The F_1's is crossed in a convergent crossing program to incorporate multiple parents (column-2). The F_1 of the multiple crosses selfed to produce F_2 population and mass selection practiced among F_2 plants. The selected F_2 plants are intermated to produce 1st selective mating series Column-3). The remaining mass selected F_2 platns are selfed to produce bulk of F_3 generation. The F_1s of the mass selected F_2 (product of 1st mating series) selfed to produce a bulk of F_2 and some are selected for intermating to form the 2nd cycle mating series (column 4). Others are back crossed to other parents not included in the orginal diallel. Thus, this system is a unique method of combining conventional breeding methods and also permits additional recombination by intermating selected genotypes.

DSM seems to be formidable due to large number of crosses and requirement of enough F_1 seeds in each crossing cycle, therefore, Jensen (1978) suggested use of male sterility factor to facilitate crossing and growing of breeding populations in a specialized environment to maximize genotypic expression of the trait under selection. This system of breeding method provides an ever-present dynamic gene pool in which selection, increase frequency of favourable alleles, intermating maximizes the opportunity of recombination, opportunity exists to adjust germplasm base by introgression at any time and useful agricultural cultivars could be extracted at any state of its evolution. Unfortunately, this method of breeding procedure has not yet been tested for its efficiency and efficacy in any crop plants and it is suggested to make use of this innovation to meet the time bound, goal oriented and diversified breeding objectives in rice.

10. SCOPE FOR THE DEVELOPMENT OF HETEROGENEOUS POPULATION

There were four waves of genetic erosion and loss of genetic diversity in rice. During the early part of the rice breeding era in India 82 research stations spread over 14 states developed 445 varieties by improving native varieties through pure line selection of highly heterogeneous farmers varieties, which is treated as the first wave. The wide adoption of semi dwarf high yielding varieties again leading to sharp reduction of genetic diversity. Hybrid approach and use of same WA cytoplasm in majority of the hybrids brought about genetic as well as cytoplasmic uniformity in rice. Increased emphasis of molecular marker assisted back crossing of mega varieties may bring down genetic diversity as the fourth wave. Besides genetic uniformity the use of intensive monoculture in the agricultural ecosystem supported by intensive crop management, maximizes the spread and evolution of particular pathogen and pests and minimizes the yield potential of crop varieties under different environmental stresses. Heterogeneous crops and cropping systems in subsistence agriculture avoids these problems but they often not been developed to maximize yield potential.

The use of planned heterogeneity in modern crop varieties was proposed by Rosen (1949), modified by Jensen (1952), and was first implemented by Suneson (1968), by releasing 'Harland' barley variety in USA. This variety emerged from his pioneering studies on bulk hybrid population and was the 28th generation seed lot of his famous composite cross bulk. To provide a combination of uniformity of agronomic traits and heterogeneity for disease resistance, Jensen (1952) proposed the use of multiline varieties. But the actual credit for conceiving a variety in a composite or synthetic form in self-pollinated crops goes to Norman Borlaug who has been instrumental in developing a comprehensive programme for producing multiline varieties of wheat (Borlaug, 1959). He proposed to blend eight to sixteen isogenic lines, each with different rust resistant allele, into a phenotypically uniform multiline variety which provides a sort of 'genetic resilience' against the disease appearing in epidemic form. Multiline breeding if properly conceived, organized and applied with different degree of sophistication can help to achieve diverse breeding objectives including high yield and stability over varied agrocliamtic conditions (Borlaug, 1981).

However, due to technical difficulties in production and maintenance of isoline mixtures and their susceptibility to non-target diseases, this excellent theory of diversification of resistance against diseases was not properly executed. Wolfe and Barrett (1977, 1980) proposed that the variety mixtures due to their alleged advantage with respect to pathological and agronomic properties can substitute the multiline and simultaneously retain the valuable features of a heterogeneous population for higher yields and stability.

Due to wide differences in agronomic uniformity and differences in various quality characteristics of the component varieties, the mixtures of varieties with several advantages over multiline were not commercially exploited. When multiline have so many undesirable side effects because of their uniformity and when lack of uniformity is the disadvantage of variety mixtures, then probably the ultimate use of intra-varietal heterogeneity would be settled with mixture of related lines (Groenewegen and Zadoks, 1979; Frey, 1982).

Besides all other advantages of growing mixtures, the hopes for benefits from mixed population mainly include improved grain yield. The expression of yield in mixtures maybe overcompensatory, giving higher yields than the expected on the basis of component cultivars; compensatory giving yields similar to the mean of their component or undercompensatory yielding less than expected is reflected by several complicated interactions between the constituent pure lines. Basically, the magnitude and direction of this intergenotypic competition reflect the feasibility of composite breeding approach to crop improvement. Studies carried out in rice and various other crops had led to the emergence of three major views on the nature of relationship between competitive ability and agronomic superiority of the genotypes in the mixed population, *viz*., no consistent relationship (Frey, 1967; Baker and Briggs, 1984); a strong positive relationship (Spitters, 1979; Valentine, 1982) and strong negative relationship (Jennings and Herrera, 1968; Khalifa and Qualset, 1974). Thus the effect of intergenotypic competition in bulk or mixed populations can be a serious problem, if the population is not managed through appropriate selection and may end up with reduced yield, vigour and plasticity.

A homogeneous composite variety constituted by mixing phenotypically similar but genotypically different related lines helps to maintain substantial amount of genetic diversity in such populations as well as expected to give longer varietal life with higher yield and greater stability of production through an optimum level of residual heterozygosity and positive intergenotypic interaction between the component lines of the population. There are several instances to demonstrate that the longevity and stability over varied environments of a variety depend on the extent the variety was homozygous at the time of its release for general cultivation. The secret behind the success of the widely adapted miracle rice IR 8 is believed to be its limited heterogeneity at the time of its release. The other convincing evidence, in particular, comes from the evolution of commercial cultivars and breeding lines developed by the University of Sydney and North-West Wheat Research Institute wheat breeding programme. The cultivar "Gamenya" which has enjoyed great popularity among farmers for a long time in many areas in Australia maybe attributed to the residual heterogeneity resulting from limited selection pressure. (Bhatt and Derera, 1973). The same theory holds

good for the popularity of many land races as compared to their pure line selections. 'Mozhagohukulu' and 'Kartikai Samba' in their native forms, for instance, are preferred by the farmers of Andhra Pradesh and Tamil Nadu as compared to their pure line selections BCP-1 and ASD-7 respectively. Similarly, 'Saruchinamali' in Odisha is still popular among the farmers than its pure lines counterpart T-141 released by the Department Agriculture years back. Until this century, farmers used land cultivars which even though more or less uniform for agronomic traits often were heterogeneous for disease reaction alleles. Coffman (1977) listed 14 district pure lines of oat selected from land cultivar 'Kherson'. Derivatives of Kherson differed not only morphologically but also in reaction to major oat disease, specially stem rust. Thus, from the foregoing instances, it is obvious that limited heterogeneity in a variety maintained probably by low frequency of outcrossing among genotypes of the population, confers to it a sort of plasticity or a sort of genetic resilience to adopt itself to varied agro-climatic conditions.

Therefore, an attempt has been made to synthesize heterogeneous populations in rice by Das et al. (1990, 1998) Hasan and Siddiq (1990, 1995) by blending different morphologically similar lines that would interact harmoniously, an equilibrium between competitive ability and agronomic productivity can be realized. The synthesized populations, either in the form of mixture or bulks, and the progenies derived through pedigree selection indicated that although the mixtures were found to be superior to pure lines and the third cycle derived bulk (bulk or the selected fixed progeny rows) over the original base population (Fig.9) indicated that there is enormous scope for intensive study to explore the possibility

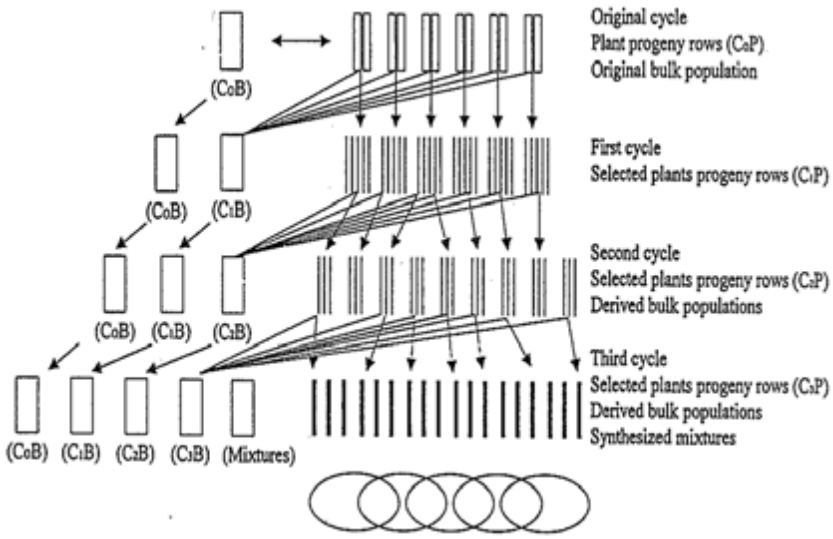

Fig. 9 Heterogeneous populations in rice

of exploitation of heterogeneous populations for realizing higher yield, greater stability of production, better agronomic uniformity and longer varietal life.

Considering the importance of heterogeneous populations for higher yield and stability a new selection approach is suggested which would combine both bulk population and pedigree mode of breeding systems to develop homogeneous mixtures for high yield and greater stability. The development of heterogeneous populations as a part of rice breeding programme as suggested would in no way be in conflict with production of new single pure line varieties but on the contrary would supplement such a programme. The prospective elite lines at the termination of selection cycles not only provide components for synthesizing heterogeneous population but also can be further evaluated and released as a highly homozygous and homogeneous variety.

11. CONVERGENCE OF CONVENTIONAL AND MOLECULAR BREEDING

Significant progress in breeding of submergence tolerant rice varieties has been achieved during the last decade due to the identification of a major quantitative trait locus (QTL) named SUB1. This gene was subsequently incorporated with a high level of precision using marker assisted backcrossing (MABC). As many as 8 mega varieties of Asian origin e.g., Swarna, Samba Mahsuri, CR 1009, IR64, PSBRc 18, BR 11, Ciherang and TDK that have been "upgraded" with the SUB1 and several others are currently being improved (Collard et al., 2013, Singh et al., 2015). The varieties with SUB1 were comprehensively tested in the field, and no negative effect of it on other traits including yield under favourable conditions (Sarkar et al., 2009). Furthermore, mega-varieties with SUB1 have been widely accepted by farmers (Mackill et al., 2012; Collard et al., 2013; Dar et al., 2018). Currently, International Rice Research Institute (IRRI), Philippines and different National Agricultural Research System (NARS) are collaborating together to develop submergence tolerant rice varieties using MABC for Africa and Asian countries like Bangladesh, Cambodia, India, Nepal, and Pakistan (Collard et al., 2013; Singh et al., 2015).

The submergence tolerant gene SUB1 generally confers tolerance to flash-flood for up to two weeks, and does not bestowed any tolerance to stagnant flooding or tolerance to anaerobic germination. (Ismail et al., 2009; Singh et al., 2011; and Kato et al., 2019). The molecular breeding efforts resulted in the introgression of anaerobic germination potential QTL *qAG9-2* in a SUB1 background variety Ciherang-Sub1 which improved the germination stage oxygen deficiency (GSOD) tolerance without sacrificing submergence tolerance strength (Toledo et al., 2015). As the genetic mechanism controlling tolerance to submergence and stagnant flooding behave in an antagonistic way, therefore,

combining these two traits require precise conventional breeding approach along with molecular breeding. Therefore, if the breeding objective is to combine a set of above-mentioned traits that make a variety unique or ideal to a given situation, 'convergent breeding' approach is the most appropriate strategy (Siddiq, 2013). The ongoing world-wide effort to breed varieties for major rice ecologies require stepwise accumulation of desirable characteristics that would combine high and stable yield, wide adaptability and acceptable quality. As marker assisted selection /backcross breeding is not enough to improve yield ceiling, therefore, there is a need to exploit new germplasm, develop new, precise and efficient molecular markers and develop efficient phenotyping methods which would be essential to enhance breeding efficiency and complement conventional breeding efforts (Collard et al., 2013).

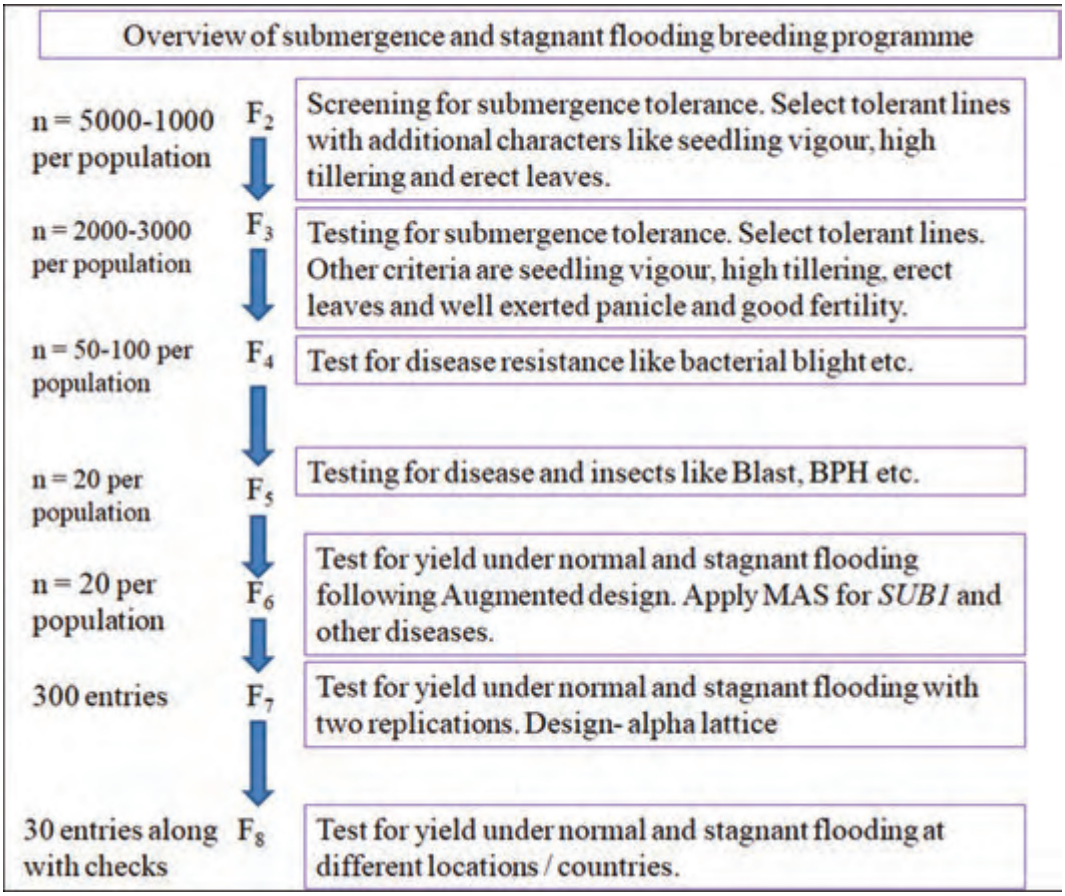

Fig 10 Flow Chart of breeding method employed to integrate Submergence tolerance with tolerance to Stagnant flooding in rice

Limited elongation ability along with stem strength/resistance to lodging imparts tolerance to SF (Kuanar et al., 2017; Kato et al., 2019). Besides, new breeding lines must also possess desirable agronomic and quality traits, and possess resistances to important diseases and insects' pests. Understanding the above-mentioned traits into consideration, a flow chart of breeding programme to integrate submergence tolerance and stagnant flooding has been outlined by Collard et al. (2013) and Das (2013) (Fig. 10).

To develop new breeding lines pedigree method with some alterations can be followed. Testing for submergence tolerance at controlled conditions is done at F_2 and F_3 stage. After screening of F3 populations, single plant selection is performed from surviving plants. From F_4 to F_6 the materials are tested for resistances to disease and insect. Testing for bacterial blight starts at F_4 while green leaf hopper (GLH), brown plant hopper (BPH) and blast are tested at only at F_6 stage. Testing of yield is started under normal and stagnant flooding conditions at F_6 stage and later on with appropriate checks cultivars. MAS are applied at F_6 stage. At F_8 seeds can be distributed to different testing stations/ countries for suitability of the locations. Applying MAS at early stages is avoided to reduce the cost. These approaches take care of submergence and stagnant flooding tolerance along with diseases and insect problems.

12. CONCLUSION

Rice breeders have been very successful in selecting for a wide range of traits using only conventional methods. Breeders do not need to use markers for every trait, even if perfect markers are available. The full advantage of MAS can be realized when markers are used to do something that cannot be done using conventional methods such as marker-assisted backcrossing (MABC) or marker-assisted pyramiding. Many breeders may not understand topics in molecular breeding in sufficient detail and molecular geneticists often do not understand breeding processes to identify the most strategic stage to apply markers. This also prevents integration between breeding and molecular geneticists including sharing of germplasm such as mapping populations or sharing of information on trait data. The breeders usually focus on developing new varieties whereas molecular geneticists are focus more on gene/QTL discovery and ultimately publications. Furthermore, the location of breeding and molecular genetics research is often separate, and may occur at different institutions.

In order to take advantage of available genomics resources in rice for molecular breeding in the future, breeders will need: Planned and well-executed QTL mapping experiments, QTL and marker validation activities, efficient and cheaper genotyping and phenotyping systems and breeder-friendly analytical tools. Integration between breeders, plant physiologists and molecular geneticists

will be critical to develop new varieties using markers. Collaboration among different partners can help in developing multiple stress tolerant rice varieties with shortest time period. (Collard et al., 2016)

While there are indeed many difficult challenges ahead in the 21st century, it is important to remember the importance of minor yield increases: 1% yield increase of a widely-grown variety will have a huge impact. Even in the advent of new promising technologies such as DNA markers, the time-tested conventional breeding and selection approaches will still account for significant improvement in increasing rice yields. A sound theoretical knowledge coupled with considerable hard work and long hours in the field are the secrets to breeding success, and this is likely to be the case for many years to come. The success and sustenance of rice breeding tells that there is no easy way to improve rice production; it demands patience, dedication, continuity and our total physical and mental commitments to field work (Das, 2013).

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Utilization of Genetic Resources including wild species for Rice Improvement

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SUMMARY

Rice breeding programs in the way of meeting demands of food security in terms of total production and nutritional quality mainly depend on exploring genetic resources to find novel useful genes for different traits. In this prospect, the rice genetic resources collected and conserved is gaining importance as a treasure of novel genes for important breeding programs. The traditional rice varieties and their wild relatives preserved in gene banks constitute a valuable gene pool for many resistance gene sources to different biotic and abiotic stresses. Modern molecular and tissue culture techniques removed the barriers of gene transfer through inter-specific hybridizations and hasten the process of rice improvement. Traditional cultivars and wild species serving as source of novel QTL/genes are better adapted to different environment and offer resistance to many biotic and abiotic stresses. Making such high valued resources available to breeders working on certain specific problems is most important. In this milieu, an effort has been made in this chapter the importance of germplasm activities starting from exploration, acquisition, collection, characterization, evaluation, documentation and conservation to utilization for accelerated improvement of rice for future food and nutritional security has been discussed.

Keywords: germplasm, hybridizations, genetic resources, QTL/genes, rice

1. INTRODUCTION

In view of the global food security, plant genetic resources (PGR) stored in gene banks at regional, national and international levels across the world bear great hope. Many desirable traits sought by breeders are found in uncharacterized accessions stored as diverse germplasm collections help swiftly respond to a new plant breeding goal or to a new stress resistance breeding in the era of climate change. Apart from that, the rice genetic resources collected earlier serve as basic raw material for initiating breeding program by the needy breeders. These resources serve as primary building blocks for needed genetic variation for many complex traits. Plant genetic resources in rice breeding attained most crucial stage as advanced breeding strategies narrowed down the genetic base of cultivated rice. The continual expansion of modern rice cultivars including hybrid rice led to massive replacement of traditional cultivars. The unknown potential of genes

hidden in unexplored sources is highly useful in future days. It is not possible to create the lost genetic resources through any of the available advanced biological methodologies; hence conservation and judicious utilization of these resources should be the way forward (Gautam et al., 2004). Maintenance of genetic wealth of rice is also important for functional rice agro-biodiversity of different rice eco-systems (Swift and Anderson, 1994).

Rice genetic resources comprise of traditional local cultivars, landraces, farmers' varieties, modern cultivars, hybrids and elite breeding materials developed by utilizing advanced plant breeding methodologies. They also include wild rice progenitors and wild relatives which can be utilized for human benefit. For several traits like resistance to diseases and pests, quality parameters and stress resistance, wild species offer an excellent source of reservoir (Tanksley and McCouch, 1997). Precise transfer of segments of wild rice genome to cultivated species by overcoming all the barriers is made possible through advanced breeding, biotechnology tools and methods (Brar and Khush, 2018). The modern biotechnological approaches and tools have come handier in using wild species much more precisely in gene transfer from wild to cultivated rice. Also, rice breeding has been changing dynamically with the invent of new molecular tools for the benefit of mankind (Patra et al., 2020). Hence, continued collection of wild forms of rice along with germplasm from unexplored sources enhances the genetic base of rice in development of new varieties. Along with conventional breeding approaches, the application of wide-hybridization in exploiting wild species and better utilization of already available genetic diversity from gene bank collections upon characterization with high-throughput phenotypic and genetic tools would constitute successful approach (Khush, 2013).

2. THE GENUS *ORYZA*

The genus *Oryza* includes two cultivated and 23 wild rice species (Sanchez et al., 2014). The genus *Oryza* contains 11 different types of genomes including diploid genomes (six) and tetraploid genomes (five) (Ammiraju et al., 2010; Stein et al., 2018). The genomes of diploids are designated as n = 12: AA, BB, CC, EE, FF and GG, where AA genome alone consists one-third of species of *Oryza* and it forms the primary gene pool of rice which allow easy transfer of useful genes among species (Vaughan et al., 2003). Among the cultivated rice species, *O. sativa* is high yielding, pantropic and is grown widely across the world whereas *O. glaberrima* is low yielding, endemic and confined to West African countries. However, the centre of origin and centers of diversity of these two cultivated rice species are found to be different. According to genetic diversity, historical evidences, archaeological supports and distribution pattern; valleys of Yangtze and Mekong rivers were concluded as the primary centre of origin for *Oryza sativa*. Foothills of

the Himalayas, north eastern states of India, Myanmar and Thailand, Yunnan Province of China are designated as secondary centers of diversity of Asian cultivated rice. It is also believed that the upland, short duration as type of rice having drought avoidance mechanisms is originated in Koraput of northern Eastern Ghats of India. On the other hand, *O. glaberrima* is believed to be originated in the inner delta of Niger River of coast of Africa (Chang, 1976; Oka, 1988).

Among different species of genus *Oryza*, *O. rufipogon/ O. nivara* and *O. longistaminata/ O. barthii* are very close relatives to the cultivated species of rice and believed as progenitors of two cultivated rice. *O. rufipogon/ O. nivara* grows wild in Asian and southeast Asian countries including India whereas *O. longistaminata/ O. barthii* is found in northern parts of tropical Africa. A study by Samal et al. (2018) confirmed that these two wild species carry AA genome. Wild types found in Asian countries and African countries are believed to have contributed to evolution of cultivated rice cultigens, whereas, wild types sharing AA genome found in Australia and Latin America also were not evolved into cultivated types due to wetland agro-ecology existing in these regions (Chang, 1987). Considering all these evidences, the genus *Oryza* is consisted of several taxa of which cultivated species are diploids (2n=24) and wild types include both diploid and tetraploid forms. Some of the species are very closely related and overlapped with other taxa forming species complex. Several species like *O. angustifolia, O. coarctata, O. perrieri, O. subulata* and *O. tisseranti* were formerly placed under the genus *Oryza* and later they have been re-assigned to different genera. The information on known *Oryza* species complex and their genome information are provided in Table 1.

Apart from cultivated rice and the wild rice, another category of rice associated with genus *Oryza* is weedy rice. The natural dispersal or natural transfer of genes among different wild and cultivated resources led to evolution of a hybrid form or hybrid swarm of rice called weedy rice. Scientifically it is known as *Oryza sativa* f. *spontanea* in Asian rice and *O. stapfii* in African rice. However, the weedy rice is always accompanied with the cultivated rice at all rice growing parts of the globe (Mortimer et al., 2000). Even though weedy rice is scientifically classified under cultivated rice (*O. sativa*), it is well characterized by its growth habit, more number of tillers, shattering character and longer dormancy. Most weedy rice types possess red pericarps and also known as red rice (Gealy et al., 2003), but few of them found to have white pericarps (Arrieta-Espinoza et al., 2005). Long-term sympatric association and distribution between wild and cultivated rice led to evolution of weedy rice through natural hybridization and introgression (Chen et al., 2004). Weedy rice also possesses very high level of genetic variability for many economic important traits and they are also considered as rich source for some economically important traits (Green et al., 2001). Apart from advantages

Table 1. Different taxa under genus-*Oryza* and their genomes (modified from Vaughan, 1989)

Sl. No.	Species complex	Taxa	Genome
1	O. *ridleyi*complex	O. *schlechteri*	Diploid
		O. *brachyantha*	FF
		O. *longiglumis*	Tetraploid
		O. *ridleyi*	Tetraploid
2	O. *meyeriana*complex	O. *granulata*	Diploid
		O. *meyeriana*	Diploid
3	O. *officinalis*complex	O. *officinalis*	CC
		O. *minuta*	BBCC
		O. *eichingeri*	CC
		O. *rhizomatis*	CC
		O. *punctata*	BBCC, BB
		O. *latifolia*	CCDD
		O. *alta*	CCDD
		O. *grandiglumis*	CCDD
		O. *australiensis*	EE
4	O. *sativa* complex	O. *glaberrima*	A^gA^g
		O. *barthii*	A^gA^g
		O. *longistaminata*	A^lA^l
		O. *sativa*	AA
		O. *nivara*	AA
		O. *rufipogon*	AA
		O. *meridionalis*	A^mA^m
		O. *glumaepatula*	$A^{gl}A^{gl}$

of these weedy rice types, they are identified as most notorious weeds in cultivated rice fields across the globe. Specifically, this problem is more persistent in direct seeded rice production system and causes significant yield loss. Thus, strategic breeding plans could help in the exploitation of these weedy rice types in improvement of cultivated rice.

3. EXPLORATION, COLLECTION AND CONSERVATION OF RICE GERMPLASM

Germplasm exploration programme in rice is a fascinating study and attract many researchers across the globe, besides, many naturalists, travelers and plant hunters also have made significant contribution in the field of rice germplasm resource collection. Rice genetic resources collected and maintained at regional, national

and international gene banks are considered as hope for future food security at global level. Collection and maintenance as *ex situ* conservation of more than seven lakh accessions of different field and horticultural crops across the world by the international gene banks of CGIAR centers helped to exchange of material for research purpose over years (CGIAR report, 2019). These global collections met the need of breeding programs that ushered green revolution in many developing countries. Among the world major rice gene banks, International Rice Research Institute (IRRI) has conserved 140,000 diverse rice accessions collected from 120 countries for human benefit (IRRI, 2016). China has several rice germplasm collections but the collection at Beijing has over 50,000 accessions. The Japanese national rice collection contains about 26,000 accessions. The USDA-ARS rice collection has 17,279 accessions from 110 countries. Because of its geography, diverse ecosystems and heritage of agricultural richness in India, the country is immensely rich in rice genetic resources and has 109,000 accessions conserved in the National Gene bank of NBPGR at -18^0 C and with 3-4% RH. The National Rice Research Institute (NRRI) devoted and made significant success in collection and conservation of more than 35,000 germplasm accessions which were deposited in long term storage of NBPGR, New Delhi.

4. CHARACTERIZATION AND DOCUMENTATION

The germplasm resources collected from its native source need to be characterized for understanding the importance/value of that resource. Characterization is the detailed study of rice germplasm for description of the plant as per the descriptors and involves determining and documenting expression of morphological, biochemical and molecular features specific to them. These descriptions can be utilized to dissect and understand the genetic diversity of collected germplasm. Germplasm collection programmes should always be well planned and duration of exploration should be well defined so as to capture the real-time evolutionary variations from specific location. NRRI has a mandate to collect the trait specific rice germplasm every year and conserve them in the gene bank. The collected germplasm characterized and studied for special characters are incorporated in breeding programs. NRRI is also involved in material exchange and sharing germplasm to the needy rice breeders across the country. Many breeders request NRRI for important germplasm accessions/donors/elite lines for special breeding programs and receive the material-on-material transfer agreement (MTA). In the past five years NRRI gene bank has shared nearly 20,000 accessions to breeders of different institutes/research stations of the country including the NRRI rice breeders for utilization purpose.

5. FIGS FOR TRAITS SPECIFIC ACCESSION IDENTIFICATION

Thousands of germplasm accessions conserved in gene bank should be evaluated and donors be identified which need to be utilized in breeding program. Given

the size of the collections at gene banks, screening of germplasm and identification of specific genotype searching is like a needle in the haystack. Testing of all the resources of a gene bank for a particular trait is very tedious, resource consuming and unscientific. Many strategies have been employed for reducing the quantum of resources tested at a time for a specific trait; core collection (Qui et al., 2013) and trait specific (Upadhyaya et al., 2013) identification are some among them. For potential use of appropriate germplasm for crop improvement, a trait-based approach, focus identification of germplasm strategy (FIGS) is getting popularity in recent times. It uses specific environment and evolutionary pressures of the environment on trait from which it is collected and helps rapid identification of large germplasm stored in gene banks for identification of potential breeding material. FIGS combine prior developmental information of the trait and environment data and quantify the relationship between them to generate the specialized knowledge on trait-based genotype utilization (Mackay, 1995; Mackay and Street, 2004). FIGS is based on the strategy that involves adaptive traits expressed in an accession may reflect its selection pressure in the environment from where it is originally sampled (Mackay et al., 2005). However, FIGS approach is considered as emerging trend in germplasm research that need to be utilized in the rice germplasm management and utilization for potential benefits in rice breeding.

6. GERMPLASM UTILIZATION

In the era of modernization, all the rice growing farmers are interested in growing high yielding and uniform varieties/hybrids than local cultivars. The shift in the choice of cultivars among farmers led to sweep out of highly valuable local landraces. On the other hand, shift is also resulting in narrowing down of genetic base of cultivated rice varieties making them amenable to infection of diseases and pests or susceptible to abiotic stresses. The key message is shifting from local to new cultivars is very much needed to achieve the yield targets and simultaneous research is required to incorporate genes responsible for resistance to pest and diseases or abiotic stresses. To achieve the goal, collecting, conserving and utilizing germplasm in the breeding programs is the need of the hour in rice germplasm research. Local rice cultivars and wild relatives are rich sources of genes for many economically important traits. In the past, many genes have been incorporated in to newly bred cultivated rice varieties from local germplasm accessions and wild types.

The change in climate is introducing new problems in developing new high yielding rice varieties. Apart from resolving the problems of climate change, it is now required to breed for nutritional security to overcome malnutrition. Many rice breeders are hunting for accessions carrying different quality traits to

incorporate them in to the new varieties. Besides biotic stresses, rice crop frequently also faces problems of drought, low temperature, submergence, water-logging, salinity/alkalinity etc. These abiotic stress situations cause drastic reduction in yield and thus varieties with built-in resistance to such stresses are desirable. Gene banks have served as the source of many novel genes against many diseases and pests in the past and contributed to achieve food security.

Apart from local varieties or farmers varieties, wild rice genotypes are also becoming part of genetic resources. Wild rice types are rich source of genes against many stresses caused by biotic and abiotic agents. Breeders utilized many of wild rice types to find solutions to severe damaging pests on rice (Table 2). Genes or QTL controlling resistance to many diseases have been mapped, cloned and characterized from wild rice species.

Table 2. Some important QTL/gens identified in wild species of rice

Stress	Genes/QTL	Wild species	Reference
Bacterial blight	*Xa21*	*O. longistaminata*	Huang et al.,1997
	Xa23	*O. rufipogon*	Yinglun et al., 2006
	Xa30	*O. rufipogon*	Xuwei et al., 2007
	Xa33	*O. nivara*	Kumar et al., 2012
Blast	*Pi57*	*O. longistaminata*	Xu et al., 2015
	Pi54rh	*O. rhizomatis*	Das et al., 2012
	Pi69(t)	*O. glaberrima*	Dong et al., 2020
BPH	*Bph10*	*O. australiensis*	Zhao et al., 2016
	Bph12	*O. latifolia*	Qiu et al., 2012
	Bph15	*O. officinalis*	Cheng et al., 2013
	Bph18	*O. australiensis*	Ji et al., 2016
	Bph21(t)	*O. minuta*	Zhao et al., 2016
	Bph38	*O. rufipogon*	Yang et al., 2020
White backed plant hopper	*Wbph7*	*O. officinalis*	Tan et al., 2004
	Wbph8	*O. officinalis*	Tan et al., 2004
Sheath blight	*qShB6*	*O. nivara*	Eizenga et al., 2013
Green rice leaf hopper	*Grh4*	*O. nivara*	Fujita, 2004
	Grh5	*O. rufipogon*	Fujita et al., 2006
	Grh6	*O. nivara*	Phi et al., 2019
Small brown plant hopper	*qSbph3d*	*O. officinalis*	Zhang et al., 2014
	qSbph12b	*O. officinalis*	Zhang et al., 2014

The genetic gain in high yielding improved varieties is achieved through creating variation by wide-hybridization and utilization of their derivatives. The role of cross compatible wild types with *O. sativa* was also glaringly visible in recent past. Advances made in the field of *in vitro* technology and embryo rescue made significant contribution in developing inter-specific cross and trait transfer from wild to cultivated species. Minimized linkage drag by multiple backcrosses and marker aided backcrosses helped in gene transfer in bi-parental crosses to a greater extent. These efforts in the field of germplasm research have led to identification and utilization of many novel genes in rice breeding. The advanced knowledge on genomics further eased the systematic characterization of wild rice species and helped in discovery of new genes for target traits. The lines enriched with genes received from wild relatives through pre-breeding activities helped and will continue to help the process of enhancing genetic variation for essential traits in the way of rice improvement. Transfer of genes from alien sources with the aid of molecular markers reduce the genetic drag and ease the selection process. The advances made in tissue culture, molecular techniques and genomics offer unlimited scope for utilizing the genetic resources including wild relatives in rice development programs. Further, new evolving algorithms like FIGS need to be adapted for increasing the efficiency of selection of appropriate accession for breeding programs from gene banks.

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Rice improvement for upland ecology

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SUMMARY

Upland rice area in India is declining due to huge population pressure, rapid urbanization and allocation of upland areas for other purposes. Similar is the trend in other Asian countries and Latin America though its area has increased in Africa. Infrastructure development and more assured production from irrigated and lowland areas has make people less dependent on upland rice, which once used to the food during period of crisis. In spite several limitations significant improvement has been made in breeding for upland rice and many improved varieties with high yield potential have been developed, not only in Asia but also in Latin America and Africa. Many useful genes have been identified from upland rice germplasm which are being utilized in breeding programs across the ecosystems and countries. Though upland rice area is decreasing but the technologies developed here will find their application new areas in changing climate scenario. In the present review, progress made in three decades of breeding efforts made in Asia, Latin America, and Africa in general and in India in particular have been discussed along with future needs.

Keywords: urbanization, breeding, germplasm, rice, lowland, high yield

1. INTRODUCTION

About half of the world's population is dependent on rice as their daily diet and it provides 35-59% of the calories consumed by 2.7 billion people in Asia. Rice is the most consumed cereals by humans and about 900 million of the world's poor depend on rice for their livelihood as consumers or producers (Pandey et al., 2010). As the rice plant is semi-aquatic in nature, worldwide the major production system for rice is lowland culture, where all or major part of the crop growing season is kept under flooded. Rice has mainly two distinct types production system i.e., lowland rice and upland rice. Rice research and development efforts are mostly emphasized on lowland rice, from where bulks of the produce are harvested. But there is another type rice culture where the crop is grown under sloppy undulated, unbounded or near level field in soil with good drainage and accumulation of water is impossible. Upland rice is usually grown in systems where little or no fertilizer is applied, and is direct seeded into unpuddled, unsaturated soil. Upland rice is also grown on marginal (to rice) lands suitable for other crops like minor

millets, oilseeds, orchards and the relatively favorable semi-wet situations merging into the shallow rainfed wetlands. Most traditional upland rice varieties are low-yielding and prone to lodging, but are adapted to non-flooded soils with poor fertility. The world has about 18 million ha under upland rice with about 42% in south Asia. Upland rice makes up nearly 13% of global rice harvested area and 4% of rice production, is generally the lowest-yielding ecosystem but supports millions who are completely dependent on it for their livelihood. Nearly two-thirds of all rice area is in Asia. China, India, Bangladesh, Cambodia, Indonesia, Laos, Myanmar, Thailand and Vietnam are the major courtiers growing upland rice. India has about 6.0 million ha under rainfed upland rice, of which 5.2 m ha is located in the eastern region covering the states of Assam (0.54), Bihar (0.29), Jharkhand (0.22), West Bengal (0.84), Odisha (0.85), Madhya Pradesh (0.26), Chhattisgarh (0.58), Uttarakhand (0.07) and Uttar Pradesh (1.58).

Upland rice has a proportionately greater importance in Africa and Latin America, where it accounts for around 40% and 45% of the rice growing areas, respectively. In Asia and Africa, upland rice farmers are among the poorest in the world and their holdings are often less than 0.5 ha in size. The situation is different in Latin America since much of the upland rice production there is mechanized and farms are larger in size. (Saito et al., 2018) reported that over the last thirty years, while upland rice cultivation area has reduced in Asia and Latin America, the area in Africa has increased. Among the two production systems in rice lowland cultivation is less risky and more productive. Because of the more assured production farmers usually put more emphasis in lowlands in terms of inputs and improved varietal use. Self-sufficiency in rice production from lowland field is also one of the reasons for crop diversification in uplands. In the recent years in India different government social welfare schemes also making food grains available to the people below poverty line, reducing pressure on marginal land. The decline in upland rice area is also evident from the data available from 71 countries in Asia, Latin America and sub-Saharan Africa which indicates that upland and lowland rice account for 8 and 92 percent of total rice cultivated area, respectively (Fig.1). There are many reasons for declining in area under upland rice cultivation across the globe. The current share of upland rice area in total rice area is related to rainfall and gross national income per capita, especially in Africa, and higher share is associated with lower rice self-sufficiency at national level. However, upland rice has its own importance for some of the countries. By and large the countries with lower gross national income (GNI) per capita tend to have higher share of upland rice area in total rice area (and also have lower self-sufficiency for rice (production–consumption ratio). But this relationship is not true in all occasions and on region wise correlation analyses significant correlation was not observed, which indicate that for any particular region high

share of upland rice are does not mean poverty or food insecurity (Saito et al., 2018). Upland rice in West Africa, is mostly grown by resource-poor farmers as a subsistence crop in low-input production systems, accounts for about 40% of the total rice area and yielding only 1–2 t/ha. High weed pressures, poor fertility status of the soil along with associated biotic and abiotic factors are the main constraints of poor yield. Traditional tropical japonica cultivars and African indigenous *Oryza glaberrima* varieties are still grown by the farmers. NERICA cultivars developed combining tropical japonica and *Oryza glaberrima* at Africa Rice Center are being tried as replacement for the cultivars. Some of the improved indica upland rice cultivars from Southeast Asia with low chlorophyll content and high tillering ability were found as attractive options to improve upland rice yield under low fertility condition.

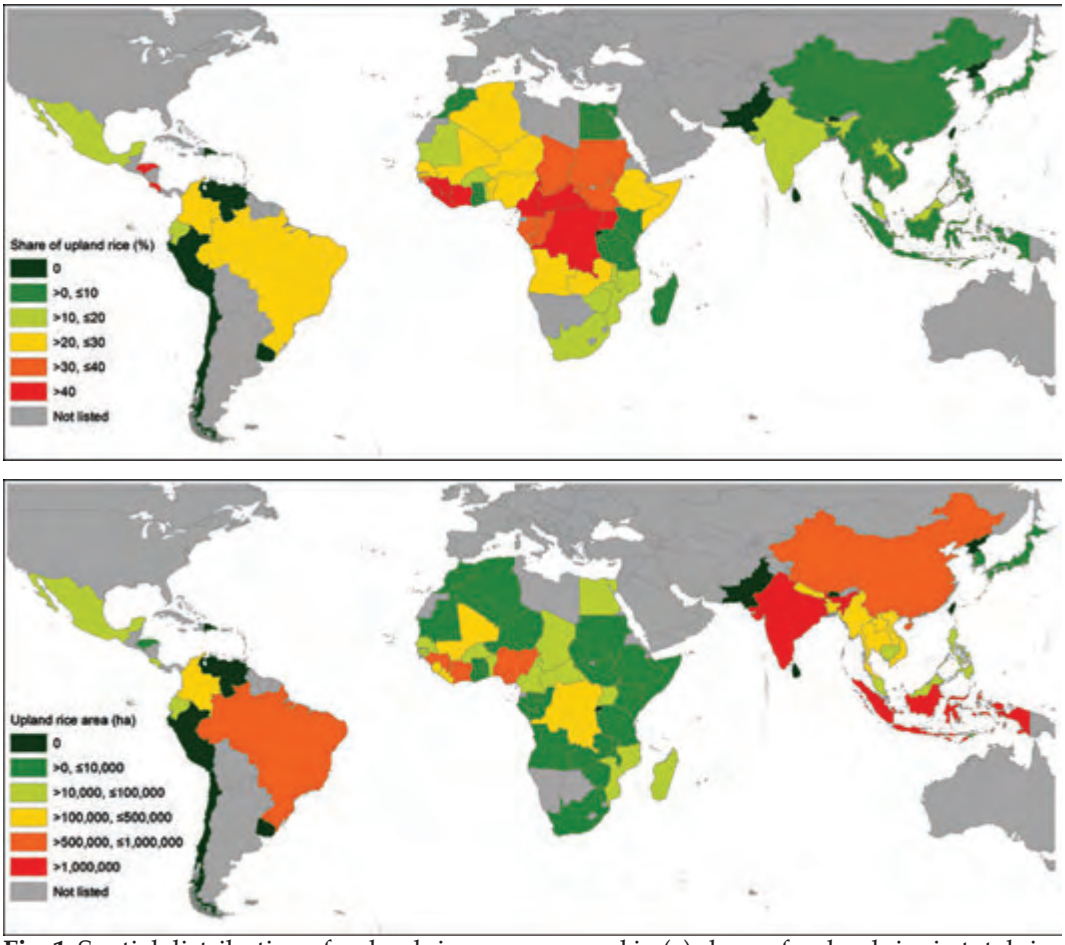

Fig. 1. Spatial distribution of upland rice, as expressed in (a) share of upland rice in total rice harvested area and (b) actual upland rice harvested area at national level. (Adopted from Saito et al., 2018)

In Latin America, particularly in Brazil upland rice is grown predominantly on large mechanized farms, in the gently undulating new croplands of the vast tropical savanna eco-region known as the Cerrado. It is an important crop in Brazil with almost 2 million ha planted every year. Breseghello et al. (2011) estimated the genetic gain of Embrapa's upland rice breeding programme over 25 years in three phases. The genetic gain was non-significant in the 1st phase (1984-1992), but a yearly genetic gain of 0.53% (15.7 kg/ha) and 1.44 % (45.0 kg/ha) reported for the next 2 phases (1992-2002 & 2002-2009), with no evidence of a yield plateau. The potential yield under water limited condition is about 3.6 t/ha though on-farm yield is around 2.0 t/ha and the yield gap is still large at 1.6 t/ha or 80% of the on-farm yield but has closed significantly from 250% in 1990.

In India, Green revolution had dissimilar benefits for different areas and the upland rice farmers were the most disadvantaged in terms of such technological benefits that eventually led to self-sufficiency in other, more favorable regions. Demographic pressures, preference of the consumers for rice and equity considerations apart, the extreme diversity of soils, topography, cultivars and climate in this ecosystem necessitated area specific approaches for overall improvement. But upland rice was never a priority in terms of research investment or infrastructure development. Worldwide degradation of the environment is a major concern in upland ecosystems though recent studies indicate that upland rice itself is more environment friendly. Usually, lowland rice fields emit annually an estimated 60 Tg CH_4 (methane) making it one of the largest agricultural sources of atmospheric methane. Methane production is known to be less in aerobic soils and dry direct seeding is reported to reduce CH_4 emission by 59-74%. Therefore, development of a sustainable environment-friendly production system for upland rice system should make it one of the favored areas for future generations of rice farmers. Bala was the first early maturing, drought tolerant, semi-dwarf dryland rice variety which was evolved at Central Rice Research Institute by hybridization and successfully adopted by farmers in the drought prone areas of Madhya Pradesh, Uttar Pradesh and Bihar. Since then, number of varieties have been developed and released by the central and state varietal release committees. Improving yield of upland rice through genetic manipulation remains a major challenge in upland rice breeding programme in India and elsewhere.

2. PROBLEMS IN UPLAND RICE

Impounding of water in upland fields is impossible even if it is bunded. As a consequence, upland rice cultivation is mostly direct seeded and grown as subsistence crop with sub-optimal input use. Productivity is very low (1.0 to 2.0 ton/ ha) and unstable. Upland ecosystem is highly diverse, as a result the crop has to face many problems. According to a survey conducted by Arraudeau (1987), major problems for upland rice are drought, weeds, blast, brown spot, stem borer

and soil depletion in major elements, particularly P deficiency. Problem of drought is synonymous with upland rice as it is mostly cultivated as rainfed crop. Depending upon the distribution of rainfall, which is often erratic, drought can occur at any stage of the crop. For a successful upland rice variety, it must have the ability to tolerate moisture stress at all stages of the growth. Seedling, tillering and flowering stages are particularly sensitive to drought. Among all stages reproductive stage is the most sensitive, seriously affect the grain filling and may cause complete sterility of the florets. Weeds are next most detrimental factors in upland rice cultivation. In upland conditions weeds are difficult to control because they emerge along with the crops, and there is a head start advantage as it is for transplanted rice. The most critical period for crop-weed competition is the first 30 days period after germination and problem of weeds can be aggravated if it coincides with drought period as herbicides cannot be applied in dry soil, even hand pulling of weeds also difficult. The typical semi-dwarf varieties are poor competitive against weeds, hence intermediate or tall cultivars are required for uplands. Blast and brown spot are the two fungal diseases cause most damage to rice production in uplands. Blast pressure is particularly high in the uplands of plateau region and hills. Rice blast is a destructive disease, which can cause a 10%-30% reduction in rice production worldwide. Blast pathogen is known for its variability and causes frequent breakdown of resistance of the varieties. The loss of resistance of cultivars against blast fungus is because of the instability of *Magnaporthe oryzae* genome and its capability of evolution towards rapid development of virulent strains when challenged with new resistant variety. Therefore, much effort in the development of durable resistant cultivars against blast and how to combat the rapid changes in blast pathogens which changes the resistance pattern is important in near future. The other important disease of upland rice is brown spot with a reported yield loss from 4 to 52%. It especially occurs in environments with limited water supply and it is often combined with imbalances in plant mineral nutrition, especially the lack of nitrogen. Normally upland soils are poor in nutrient status and water holding capacity. Due to the increase in variability in rainfall, the brown spot is becoming more severe as the drought is more frequent. Drought and nutrient deficiency have long been recognized as a key factor favoring brown spot epidemics. Early studies indicated that water shortage enhances brown spot and the disease is increasing over the year particularly in rainfed areas and the higher incidence has been reported on direct seeded rice. Host plant resistance is an effective and economical way to manage the brown spot disease however, breeding efforts are limited for the development of resistant varieties. Among the insects, Rice bug or stink bug and termite are the major insect pests in most upland environments and causes extensive damage at milk stage and crop establishment stage, respectively. Stink bug penetrate the developing grain with their sucking mouthparts and suck the

white fluid referred to as "milk". Infestation at the early stage of the grain development prevents the filling of the grain. If the attack is at the later stage the grains after being sucked by stink bugs subsequently stained by the bacteria or fungi which enter the puncture wounds, leads to grain discoloration and reduce the market price. Termites are responsible for severe reduction in plant stand in uplands. Infestations are worse in deep, light textured soils with low moisture content. Termites mostly feed on roots and germinating seeds but sometimes move to above ground parts also. Sometimes infestation of stem borers is also observed in upland rice field. Upland rice is grown in a wide range of conditions and soils are equally diverse. Most problems of upland rice soils are because of its aerobic in nature and unfavorable range of pH levels. The most common problems of upland soils are low inherent fertility, low in organic carbon and poor availability of nitrogen and phosphorus fixation. When soil is aerobic nitrogen is present predominantly as nitrate. In the semi-wet situations, with intermittent aerobic and anaerobic conditions, leaching and denitrification of NO3 further depletes the N status. Availability of phosphorus is limited due to fixation in acidic soils.

3. GENETIC RESOURCES AND DIVERSITY

At the beginning of the upland rice breeding programme, traditional *aus* cultivars grown in upland areas form the base material for genetic enhancement. These traditional varieties being cultivated by farmers for thousands of years possess several desirable characteristics required for adaptation to the target ecosystem. These landraces have evolved and are still cultivated under most harsh environmental conditions and therefore have developed and preserved tolerance mechanisms for multiple abiotic and biotic stresses. These traditional *aus* accessions are therefore highly valuable for varietal development as a source of novel tolerance traits but also for gene discovery point of view. In *aus* cultivars, early vigour, droopy leaves, deeper roots, moderate tillering and high grain weight were the preferred characteristics while indica genotypes were extensively utilized for yield attributes and grain quality traits. Tropical japonica cultivars from upland are highly vigorous with broad and thick leaves, low tillering, having deeper roots, mostly drought tolerant and high panicle weight type but were poor sources of insect tolerance. In Indian upland rice breeding programme most commonly, utilized accessions are N22, Brown gora, Black gora, White gora, Dular, C 22, Lalnakanda 41, Laloo 14, Kalakeri, Salumpikit, Ch 45, Annada, CR 143-2-2, Moroberekan etc. A list of most commonly used donors in upland rice breeding programme has been provided in Table 1. Molecular diversity of such 104 upland rice donors comprising of both traditional and improved cultivars was carried out using 43 SSR markers. All 43 SSR markers were polymorphic across the genotypes. A total of 109 alleles were detected with an average of 2.53 alleles per locus and 10 were found to be rare alleles. Out of 43 primers, only one marker

Table 1. List of most commonly utilized donors in upland rice breeding programme

Character	Aus	Indica	Japonica
Drought tolerance	N22, Kalakeri, Brown gora	Lalnakanda 41, CR143-2-2, Annada, Bala, RR 18-3, VLDT1, Sukhawan	Kinandong Patang, OS 4, OS 6, 63-83, MI 48, Moroberekan
Drought recovery	Brown gora, Black gora	CR 143-2-2, Annada, Bala, C22	UPL Ri 5
Earliness	Teenpakhia,Brown gora, Karhani	Heera, Sattari, Kalyani, Sneha, RR 145-22	IRAT 112, IRAT 10
Deep root	Kalakeri	Vandana	Azucena, OS 4 Moroberekan
Vegetative vigour	Brown gora, RR 2-6, VHC 1253	Kalyani	-
Broad leaf	Sathi 34-36, Surajmukhi	CR 143-2-2	Most upland varieties
High grain density	N22	RR 158-327	-
High grain weight	Sathi 34-36	-	IRAT 115, Khamu, Khao Khae
Aroma	HRC 410 (Brown gora)	Raskadam	Azucena, Khao Khae
Tolerant to adverse soil	Dular	Rasi, Vandana	MI48, IAC 1131
Tolerant to Blast	RR 174-1	RR 166-645, RR203-16, Rasi	Moroberekan, 63-83, OS 6, 5173
Tolerant to Brown spot	-	RR 35-4-1,RR 36-141, Ch 45	-
Tolerant to stink bug	Black gora	Sneha, RR 50-3, RR 165-1160	-

(RM 23) showed four alleles, 21 primers showed three alleles and remaining 21 primers showed two alleles. Dendrogram constructed using UPGMA resulted in 7 clusters at 1.5 linkage distances (Fig. 2). Cluster I consisted of RR 345-2, Clsuter II consisted of Salumpikit, Cluster III consisted of Abhishek, RR 218-65 and Vanaprabha, Cluster IV consisted of Sadabahar, RR 158-24, RR 348-2 and NDR 1045-2, Cluster V consisted of DDR 105, Birsadhan 101, CT 13370-12-2-M, UPLRi-7 and VLDT-1, Cluster VI consists of RR 20-5 and Azucena, Lalnakanda and Cluster VII consists of P 0013, IAC 25, B6144F-MR-6, Palawan and Tulsi.

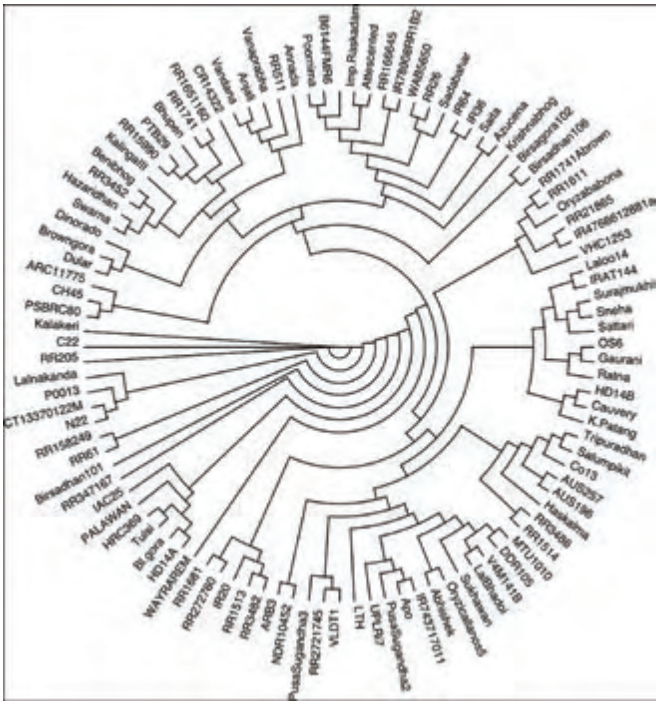

Fig. 2. UPGMA cluster showing genetic diversity among donors utilized in upland rice breeding based on SSR markers

4. PROGRESS IN UPLAND RICE BREEDING

4.1 International

At the international level varietal improvement for upland rice has progressed independently in tropical Asia, Africa and Latin America in collaboration with different international agricultural research centers (Gupta and O'Toole, 1986). Varietal improvement in Africa is mainly through international programs as there were very few national programs existed. Among the national programs, National Cereals Research Institute, Ibadan in Nigeria is the pioneer in upland rice breeding in Africa and developed many varieties among them OS6 (FARO, 11) became very popular because of its drought tolerance (Virmani et al., 1978). The Institute has specifically developed 63 rice varieties that are low N and water use efficient, pest and diseases resistant for various rice ecologies, with the recently released varieties FARO 58, 59 are for uplands. Upland rice research began in Senegal, in the 1950s and the national rice breeding program with their different partners has released up to eight upland rice varieties having a mean yield potential of around 4 t/ha (Alain et al., 2016). In the Ivory Coast the Ministry of Agricultural Research has released several useful varieties including Moroberekan, before 1966.

Later rice breeding was done in collaboration with IRAT. International Agricultural Research Centers such as IITA, IRAT, WARDA (Africa Rice now), IRRI began large scale testing of new varieties in 1976. These IARCs extensively collaborated among themselves and develops and provides superior breeding lines to the national programs to enhance their rice production. The varieties developed at IRAT are known for their deep root characters for drought tolerance. The IITA varieties had good levels of drought and blast resistance and also better tolerance to acid soil. The varietal development program in Africa revolutionized with the development of NERICA rice (Jones et al., 1997). The New Rice for Africa (NERICA) is a new variety of rice developed by the Africa Rice Center (WARDA) from a cross between the local African rice *Oryza glabberima* and the Asian type *Oryza sativa*. It is becoming increasingly popular amongst farmers for its early maturity, drought and pest resistance. Seven NERICA varieties, which were popular among farmers, were released in 2000 by WARDA. In 2005, eleven more NERICA varieties were released by the Africa Rice Center (WARDA) Variety Nomination Committee, based on their excellent performance and popularity among farmers bringing the total number of upland NERICA varieties released for cultivation to eighteen. All these 18 NERICA varieties are suitable for the upland rice ecology of sub-Saharan Africa. The most important aspect is that the yield of NERICA in the normal cropping season is exceedingly high. The average yield of 2.1 tons per hectare is twice as large as the average in sub-Saharan Africa. Development of NERICA rice opened up new gene pools and increased rice biodiversity to scientific community.

In Latin America, upland rice improvement program was initiated in Brazil at the Instituto Agronomic Campinas (IAC), São Paulo. This institute is concerned about rice production since 1937, and the first successful hybridization in Brazil was done in February, 1938 (Germek and Banzatto, 1972). Since then, a large number of important cultivars have been released by the Rice Breeding Program. Until 1989, most of upland areas of Brazil were planted with varieties IAC25, IAC47, IAC164, and IAC165, developed at this institute (Guimaraes 1993). Upland rice breeding program at the National Rice and Beans Research Center (EMBRAPA/CNPAF) began in 1976 combining IAC developed varieties with African germplasm 63-83, OS6, and LAC23 to broaden genetic base and new varieties Cuiabana, Araguaia and Guarani were released in 1985 and 1986. The varieties developed during the first phase (1984-1992) of the breeding were suitable for low input farming having characteristics of traditional germplasm (Breseghello et al., 2011). In the second phase (1992-2002) there was strong collaboration with CIAT and United States and focus changed towards the development of varieties suitable for high input, fully mechanized cropping system where lodging tolerance is a priority in addition to superior grain quality. This phase resulted into the

development and release of seven varieties which include Progresso, Maravilha and Primavera, landmark varieties for Brazilian uplands. The current phase of Embrapa's breeding program focuses on selection grain yield while keeping other characters in suitable range. The genetic gain for grain yield in the period of third phase (2002-2009) is 315.0 kg/ha which is 2/3 of the total gain in the 25-years period was achieved (Breseghello et al., 2011).

In south and south-east Asia, International Rice Research Institute (IRRI) collaborated with national programs of different countries (Bangladesh, Combodia, Indonesia, India, Thailland, Philippines etc.) to help in identifying more productive cultivars. In the early part, upland rice breeding in most of the countries was limited to collecting farmers' varieties, purification, and evaluation with limited hybridization. During this period many improved varieties from tall parents were released. Some of the prominent varieties of that period are Dular, Kataktara, Dharial, Panbira, Marichbati at Bangladesh; Palawan, Azucena, Dinorado, MI-48, BPI-1-48 at Philippines; Sew Mae Jan, Goo Muang Luang and Dawk Payom at Thailand; Seratus Malan, Genjah Lampung, Pulut Nangka, and Leter at Indonesia. Upland rice breeding in the Philippines began early than any other countries and released varieties (Milpal 4, HBDA-2, BPI-1-48 (M1-48), and Azmil 26) developed through hybridization. Later in the 1970s, the University of the Philippines at Los Baños developed C22, UPL Ri-3, UPL Ri-5, and UPL Ri-7 which have intermediate height, moderate tillering ability, and good grain quality. Some of these varieties were introduced in other countries though INGER program of IRRI and utilized by the breeders for varietal development under national program. Upland rice improvement program at IRRI progressed in collaboration with national programs and international institutes such as IITA, WARDA, IRAT and CIAT (Gupta and O'Toole, 1986). Larger numbers of crosses were made at IRRI involving improved germplasm and traditional varieties and distributed to partners for location specific selection. Several lines with good yield potential in favourable areas and moderate drought tolerance were identified. IRRI also established a mass screening technique for vegetative and reproductive stage drought tolerance (Chang et al., 1974) and correlation of root and shoot traits in upland rice with drought avoidance/ recovery ability was identified (Chang et al., 1984) for selection as secondary traits. Use of secondary traits for the improvement of drought tolerance in terms of grain yield under stress met with limited success because of several limitations (Tuberosa et al., 2002). From the first decade of this century IRRI started using direct selection for grain yield under stress for the improvement of drought tolerance. It has been demonstrated at IRRI that if the stress is applied uniformly and consistently, yield under stress can be as heritable as yield under control condition and resulted in significant gain in upland rice drought tolerance (Bernier et al., 2007; Atlin et al., 2004; Venuprasad et al., 2007;

Kumar et al., 2008). For a more intensive high input upland system a new set of cultivars were developed for Asian tropics with improved lodging resistance, harvest index and input responsiveness (Atlin et al., 2006). These cultivars combined yield potential of high yielding lowland rice with aerobic adaptation of upland rice holds promise for water limited environment. To overcome major constraints of the uplands, the IRRI upland rice program has covered the whole spectrum from pre-breeding understanding, trait development to specific stresses and identifying, locating and mapping of relevant genes/QTL for deployment, and developing an appropriate methodology from marker assisted selection.

Drought is the major constraint for upland rice (Widawsky and O'Toole, 1990) and since beginning upland rice breeding has become synonymous with breeding for drought tolerance. Drought is considered as one of the most complex and toughest stresses to overcome, particularly in rainfed uplands and poorly irrigated areas (Reyes, 2009). Around 23 million hectares of rainfed rice growing area are estimated to be drought prone in South and Southeast Asia of which 10 mha belongs to uplands (Pandey et al., 2007), costing 17–40% yield reduction in severe drought years. Numerous attempts have been taken to develop drought-tolerant rice, but resulted in poor success. Earlier molecular genetic analyses identified numerous QTL (quantitative trait loci) of secondary traits such as root architecture, leaf water status, panicle water potential, osmotic adjustment and relative water content, etc., but failed to identify any major genes that regulate these traits; mainly due to low mapping resolution and weak phenotypic effect. Rice breeding for drought tolerance considering the secondary traits such as root architecture, water use efficiency, etc. remained unsuccessful (Babu et al., 2003; Lanceras et al., 2004). It has also been believed that grain yield as a selection criterion is not suitable in breeding rice for drought tolerance because of the complexity nature of this trait. Several experiments to standardize the procedures for uniform screening of segregating populations for grain yield under reproductive-stage drought (Venuprasad et al., 2007, 2008; Kumar et al., 2008, 2009) showed moderate heritability of grain yield under drought, thereby confirming the suitability of grain yield as a selection criterion. Once screening protocols were standardized, large-scale conventional breeding and quantitative trait locus (QTL) identification programmes were started, using yield as a selection criterion. Drought research at IRRI over the past decade has concentrated on direct selection for grain yield under drought. This approach has led to the successful development and release of 17 high-yielding drought-tolerant rice varieties in South Asia, Southeast Asia, and Africa.

Using grain yield under reproductive-stage drought as a selection criterion, around 14 large-effect QTL for grain yield (*qDTYs*) under reproductive-stage drought for both upland and lowland conditions have been identified (Kumar et

al., 2014). Six of these (*qDTY1.1*, *qDTY2.2*, *qDTY3.1*, *qDTY3.2*, *qDTY6.1*, and *qDTY12.1*) showed an effect against two or more high-yielding genetic backgrounds in both the lowland and upland ecosystem, indicating their usefulness in increasing the grain yield of rice under drought. The *qDTY12.1* was the first reported large-effect QTL for grain yield under reproductive stage drought (Bernier et al., 2007). Physiological characterization of *qDTY12.1* revealed an effect of the QTL on water uptake (Bernier et al., 2009).

The difficulties of reliably screening for drought tolerance in the field make marker-assisted breeding (MAB) an attractive alternative. Therefore, integration of conventional and molecular breeding techniques could be an efficient and fast-track approach to develop drought tolerant varieties combining higher yield potential. MAB has been undertaken to improve grain yield of popular rice varieties such as Vandana, Anjali, IR64 and Swarna. Several *qDTYs* (*12.1, 3.1, 4.1, 2.2* and *1.1*) have been targeted for MAB. For instance (Henry et al., 2015) reported that a pyramided rice line with *qDTY2.2* + *qDTY4.1* showed the greatest degree of improvement under drought compared with IR64 in terms of yield under stress (Kumar et al., 2014). Scientists have reported the loss of the *qDTY1.1* allele during the green revolution was due to its tight linkage in repulsion with the *sd1* allele. Other drought yield QTL (*qDTY)* also showed tight linkage with traits rejected in green revolution varieties (Vikram et al., 2015).

Differentially expressed proteins (DEPs) under drought were identified using isobaric labeling approach and MapMan-based analysis. DEPs were involved in photosynthesis, respiration, energy generation, and carbon–nitrogen acquisition/remobilization, largely through the pathways of sugars/starch and amino acid metabolism. DEPs could be related to drought-specific morpho-physiological responses of 'Vandana' NILs (near isogenic lines) introgressed with drought QTL $DTY_{12.1}$ (Raorane et al., 2015).

4.2 National / NRRI

In, India systematic rice improvement program began when Central Rice Research Institute (CRRI) was established at Cuttack in 1946. Attempts to develop varieties by hybridization to withstand drought began in Tamil Nadu and Kerala in 1955. The drought resistant variety Co 31 was released from Coimbatore, Tamil Nadu (Rajagopalan, 1966) but this variety had long growth duration and photoperiod-sensitive. Richharia and Govindaswami (1966) listed more than 150 improved tall varieties with varying maturity duration (80-110) for upland cultivation developed in different states during first half of the 19th century. Upland rice improvement programs in their earlier phases primarily involved the isolation of pure lines of land or popular local varieties by single plant selection. For example, N22 was selected from Rajbhog in U.P', BR 19 from Brown gora in Bihar and PTB

10 from Thavala Kannan in Kerala. These varieties had low tillering capacity, weak stems, long panicles, coarse grain and lodge with applications of fertilizer but the farmers preferred them for growing in unfavorable uplands because of stability. The All India Coordinated Rice Improvement Project (AICRIP) initiated in 1965 organized a coordinated rice improvement program involving state agricultural university/ department breeding programs with those of CRRI and other ICAR institutes and ushered into a new era of rice breeding.

At National Rice Research Institute (known as CRRI earlier) upland rice breeding was initiated with the evaluation of existing early varieties for adaptability in dryland areas with deficient moisture regimes (Murty et al., 1967; Bhattacharjee et al., 1971). They identified varieties such as Lalnakanda 41 and CH 45 having better drought tolerance at the seedling and tillering stage, while MTU 17 could stand moderate stress even at the flowering stage. The focus of research at central institute shifted to development of shorter duration (90-110 days) varieties to fit in the southwest monsoon season. Semidwarf gene was first used in upland varietal improvement in India in 1965. CR 42-38 (Bala) from the cross N22/TN1 was released as a promising for direct seeded dryland conditions (Rao and Chaudhary, 1969). A series of early duration semi-dwarf varieties which included Annapurna (Kerala), Cauvery (Tamil Nadu), Parijat (Orissa), Pusa 2-21 (IARI), Rasi, Kanchan (Bihar) and Annada were released. Later on it was realized that semi-dwarf varieties are not suitable in unfavorable uplands, rather varieties with tall stature are more appropriate to challenge the high weed pressure and first semi-tall variety Kalinga III released in 1983 from CRRI. The momentum of upland rice varietal improvement programme was intensified with the establishment of Central Rainfed Upland Rice Research Station at Hazaribag under Central Rice Research Institute during early 1980's (Chaudhary and Rao, 1982), Strategies for breeding drought tolerant rice varieties were discussed in depth at a 1978 symposium and a 1980 group meeting held at CRRI on research priorities in rice improvement for drought prone areas and at DRR, Hyderabad in 1983 and 1989. These discussions suggested a number of activities for development of appropriate technologies related to varietal development for different categories of rainfed upland rice. Development of varieties using drought tolerant *aus* and high yielding *indica* genotypes was undertaken at CRURRS, Hazaribag and released its first drought tolerant variety Vandana in 1992 as an early duration (90-95 days), tall, drought and blast tolerant culture with good weed competiveness and yield (2.5 t/ha) (Sinha et al., 1994). Vandana, which is highly drought tolerant and adaptable to upland situation, was developed from one of such crosses (C22 /Kalakeri). C22 is a semi tall, medium duration variety from the Philippines, and Kalakeri is tall, drought tolerant, traditional cultivar from Orissa. Further efforts led to the development of varieties like Anjali (2002),

Virendra (2006), CR Dhan 40 (2008) and Sahbhagi dhan (2011). Simultaneous efforts at IGKV, Raipur and NDUA&T, Faizabad led to the development and release of lndira Barani dhan -1 and Shushk Samrat, respectively. To address the issue of poor adoption of improved varieties in uplands a participatory varietal selection (PVS) program was initiated in Eastern India, independently in collaboration with IRRI and DFID. PVS has been helpful in integrating farmers' preferences concerning grain types and relevant traits and genes into breeding programs (Courtois et al., 2001). The collaboration of DFID with Birsa Agricultural University at Ranchi for a PVS program led to the successful development of two popular upland varieties BVD 109 and BVD 110 in 2005 (Conroy et al., 2009). These earlier efforts concentrated on identification of traits (deeper root system, delayed leaf rolling and wilting) and their incorporation in high yielding varieties conventionally. An Upland Rice Shuttle Breeding Network linking centers in eastern and western India working on upland rice was initiated in 2002 under the aegis of ICAR-IRRI collaborative program to address location specificity in the deployment of genotypes by developing and evaluating breeding lines under naturally occurring stress in the target environment (Mandal et al., 2009). This network greatly facilitated exchange of germplasm and generation of information on varietal performance in a range of soil and climatic conditions with better precision. Breeding target environments for upland rice cultivar development have been delineated in to favorable (high yielding) and unfavorable (low yielding) environments and direct selection for grain yield under both moderate- and low-input conditions was advocated. The variety Sahbhagi dhan is conventionally bred using direct selection for grain yield under stress. The success of 'Sahbhagi dhan' due its resilience and higher productivity under stress motivated the research group to identify QTL that perform well under stress. Several QTL (*DTY12.1, 2.2, 3.1, 4.1, 6.1, and 9.1*) were identified at IRRI. Breeding programmes at NRRI-CRURRS, Hazaribag focused on the transfer of these QTL to productive background, in collaboration with IRRI. The first drought tolerant variety developed through marker assisted selection (IR64 Drt1), where 2 major QTL (*qDTY2.2* and *qDTY4.1*) for grain yield under drought stress have been introgressed into popular variety 'IR 64', and released in 2014. Several institutions including CRRl, lRRl, BAU and DRR were involved in phenotyping and advancement of the material through multilocation testing. The Hazaribagh center has now moved on to combine drought tolerance and blast resistance genes in productive backgrounds. Vandana has been improved for drought and blast resistance with drought QTL (*12.1*) and blast R genes (*Pi 2*). One of the QTL/gene introgression line CRR747-12-3-B possessing drought trait yield QTL, *DTY12.1, DTY2.3, DTY3.2* along with blast resistant gene *Pi2* and low phosphorus tolerance gene *pstol* have identified as promising and proposal submitted for release as variety.

5. Research needs

The multiple traits that are to be incorporated into elite high yielding varieties are governed by a combination of minor and major genes. This can be achieved using diverse parents with desired traits. Pedigree breeding method has been most widely used for upland rice improvement (Prasada Rao, 1984) but it is relatively slow and takes long time to deliver the products. In the current climate change scenario it is necessary to reduce the time of breeding cycle because the farmers who use varieties bred very recently are at least risk with respect to climate change (Atlin et al., 2017). There is need to modernize the upland breeding program with rapid generation advance, genomic prediction and recycling of new parents on the basis of field testing to increase the genetic gain. The major limitation of drought stress breeding is the lack of suitable screening methods for large volume of breeding population along with its environment specific nature. Earlier studies tried to link secondary traits with drought tolerance. However, recently direct selection for yield under drought and well-watered condition has been accepted for drought tolerance in rice (Kumar et al., 2008; Venuprasad et al., 2008; Torres et al., 2013). Recently scientists at IRRI screened rice accessions originated from 47 countries for yield under drought and well-watered conditions during 2004–2009. Among the 988 accessions, total 65 accessions (originated from 13 different countries) were considered drought- tolerant accessions based on highest grain yield in any crop season (Torres et al., 2013). Interestingly, distributions of these tolerant accessions (country of origin) clearly show that most drought-tolerant accessions (48 accessions, *i.e.*, 73% of total tolerant accessions) originated from Bangladesh and Eastern India. Many drought-tolerant rice cultivars such as Kataktara, Da2, Dular, Shada Shaita, and DA28 are still being cultivated in Bangladesh in *aus* growing season in considerable areas (Hossain et al., 2013).

Genetic improvement of upland rice for drought tolerance for both moderate stress at flowering and severe vegetative stage stress is required. QTL for drought tolerance have been identified and successfully introgressed into upland varieties Vandana and Anjali along with other varieties through a well-planned marker-assisted breeding approach (Kumar et al., 2014). Introgression lines with major QTL give a yield advantage of 0.5 to 1.0 t/ha but the magnitude of effect is background specific. To increase the magnitude of effect either we have to identify new QTL with larger effect and introgress QTL in combination. Major limitations in the use of these QTL are their specificity to genetic backgrounds and environments. It is therefore becoming important to characterize the compatibility of these QTL in different genetic backgrounds, environments and different combinations. The physiological basis of improved tolerance in these

QTL needs to be studied so that QTL with different mechanism can be combined to achieve the maximum effect.

Blast and brown spot are the major diseases in uplands and problems. These biotic stresses get accentuated under moisture stress (Variar et al., 1993). So, genetic improvement of upland rice for blast and brown spot diseases is imperative to stabilize the production. The efficacy of blast resistance is increased when genes for partial resistance is incorporated along with major genes. Two major genes for blast resistance, *Pi2* and *Pi9* have been incorporated through MAS and haplotypes of *Pi9* also have been identified from Indian landraces (Imam et al., 2016) It is necessary to develop and validate functional molecular markers for these two genes to facilitate their use in MAB. Genetics of brown spot is poorly understood. QTL have been reported for resistance against brown spot disease but none them explained large phenotypic variation for this trait. In a preliminary study we have also identified 3 QTL for brown spot resistance in Kalinga III/ Moroberekan population which need to be validated for future use. Upland soil is very poor in P nutrition and P fixation is a major limitation. Deployment of *Pstol* gene in improved varieties, which increases the ability of rice roots to take up soil phosphorus may be one option to tackle this problem. Weed is the most damaging biotic stress in direct seed rainfed uplands. Tolerance to this biotic stress has been identified in traditional and other varieties. The physiological basis and genetic control of tolerance required to be studied with a view to deploy the traits in breeding programs

6. CONCLUSION

As the area under upland rice cultivation is decreasing due to high population growth, different developmental activities and also diversification for other crops, new challenges and new constraints have to be faced. These new constraints include shortened fallow periods, declining soil fertility and increasing soil erosion, environmental degradation and more problem of drought because of climate change effect. All these issues cannot be overcome through genetic improvement alone. Adjustment in the breeding strategies, methodologies and management is the key to address the farmers' needs. Upland rice breeding program has to adopt faster breeding method to increase the rate of genetic gain, while also minimizing the operational cost (Atlin et al., 2017). RGA is one of many effective methods that can allow breeders fixed their materials quickly and coupled with genomic selection allows for predictions to be made about new recombinants faster than in traditional pedigree schemes (Collard et al., 2017). The new upland rice varieties developed for Asian tropics were shown to out-perform local check varieties for higher grain yield and harvest index and responsiveness to nitrogen fertilizer (Atlin et al., 2006; Saito et al., 2006). While HI of 0.60 is considered as an achievable

goal for semi-dwarf, irrigated rice (Swaminathan, 1988), the target for short duration genotype with larger panicles and reduced number of tillers for the uplands would be 0.50, for which the crop needs to produce 10 tha^{-1} of biomass in about 90-100 days. However, much work and progress needed to meet the new challenges faced by rainfed upland farmers of the country. In a changing environment it is an enormous challenge to produce rice in a sustainable way. Recent breeding program efforts of broadened the genetic base of the materials need to be continued. MAS need to be geared up for pyramiding of already identified large effect drought QTL in the adapted background. The physiological basis for this improved tolerance needs to be studied simultaneously with new mapping efforts. Durable resistance to blast remains a major objective and all available innovative strategies should be implemented to address this issue, including gene pyramiding or the exploitation of long-lasting partial resistance genes. Inclusion of participatory varietal selection in the breeding program would be an important strategy towards obtaining feedback on farmers' preferences for rice varieties to increase the likelihood of adoption of upland varieties.

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Genetic Improvement of Rice for Lowlands

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SUMMARY

The rainfed lowland ecology constitutes around 37% of the total rice growing area in India. Enhancing and stabilizing rice productivity under rainfed lowland ecology is imperative to improve the overall rice productivity of the country. But the genetic enhancement of rice under this ecology poses unique challenges which include occurrence of frequent drought and flash floods experienced during various crop stages. Hence the varieties for the lowland ecology have to be drought and submergence tolerant. Seed dormancy, anaerobic germination ability, lodging resistance, tolerance to pre-harvest sprouting and tolerance to major biotic and abiotic stresses are crucial traits to be deployed in lowland rice varieties. The varietal improvement programme at the ICAR-NRRI had attained enormous progress in developing varieties for the lowland ecology. Developments in the frontier areas of rice molecular biology have been successfully incorporated in the breeding programmes for developing climate smart lowland rice varieties. The updated information on genetic improvement of lowland rice, with special focus on the varietal development has been discussed in this chapter.

Key words: Climate resilience, drought, rainfed lowlands, Sub 1, submergence

1. INTRODUCTION

Rice is the staple food crop of India. It is cultivated in about 43 mha of land and 37% (16 mha) of areas are under rainfed lowland ecology. Moreover, a large area of about 51% (22 mha) is being cultivated as rainfed rice crop. Thus, this area also faces the constraints related to rainfed cultivation. About 92% of these rainfed lowland areas are located in the eastern region of the country, which has to be addressed scientifically to improve the production and productivity in this area.

Depending upon the water depth and duration of water stagnation in the lowland, the lowland rice is categorized into rainfed shallow lowland, semi-deep, deep and very deep water or floating type ecosystem. Often rainfed shallow lowland is characterized by water accumulation of about 0-50 cm. The productivity and production are very low from this ecology because of extreme types of stresses like drought, flash flood, soil toxicity problems, etc. Though the ecology possesses sufficient amount of water for rice cultivation, but occasional

drought spells do also occur. It is reported that 52% rainfed lowlands are drought prone and about 17% are flood prone. Prolonged submergence and water stagnation due to flood for more than 10 days often are encountered during the cropping season in most of the lowland areas. Further, due to rainfed situation, the scope for supplementary irrigation is very much limited. The genotypes suitable for lowland ecologies have longer maturity duration, for example in shallow lowland 140 days, for water logged areas more than 140 days (150-160) and comparatively higher plant heights. The varieties of this ecology generally require more plant height than the irrigated ecology because of water stagnation.

Due to the water level variations, soil types and duration of crop requirement, this ecology is not suitable for raising any crop other than paddy. The anticipated change in climate in future may compel the farmers to produce rice even from stressful environments. Another challenge is growing population and over-exploitation of land and other natural resources due to urbanization, the pressure on food security will continue and rise. The effect of green revolution was principally confined to irrigated rice growing areas and rainfed ecology was not shown any significant increase in production. Thus, the rainfed ecology of eastern India remained untapped for higher production. Now, growth in rice production has been almost stagnant. Therefore, the viable option for increasing the overall rice production of the country lies in improvement in productivity of rainfed lowland ecology. This ecology faces tough situation during the cropping season, with respect to kind and duration of stress. Overall, development of climate smart varieties has the potential to fight against the challenges of this ecology. Thus, there is an urgent need of region-specific climate smart breeding to replace the old mega rice varieties with stacking of various stress tolerance genes. Hence, simultaneous incorporation of drought and submergence tolerance besides imparting lodging resistance, anaerobic germination ability, seed dormancy and tolerance to major biotic and abiotic stresses becomes very crucial for developing future rice varieties for lowlands.

Many functional genes and QTL for useful traits of importance have been identified for lowland rice. Now it will be interesting to see how these QTL or genes will behave when they are all taken together in a single background. The impact of the green revolution has side-lined the eastern Indian rainfed shallow lowlands. Sizeable rice areas of eastern India are under shallow lowlands with low production. The irrigated ecology has shown symptoms of yield plateauing. Thus, this traditionally neglected ecosystem has got now tremendous potential to increase the yield level and total production as a whole. This chapter deals with the updated information on genetic improvement of lowland rice for attaining higher and sustainable productivity.

2. TRAIT SPECIFIC IMPROVEMENT FOR LOWLAND RICE

2.1 Abiotic stress

Submergence and drought are the two major abiotic stresses in rainfed rice cultivation. The shallow lowland area faces the intermittent drought during the crop growth period. The submergence is most prominent issue in lowland ecology. Both the stresses are very extreme types and may also occur in the same season which led to very severe yield losses. Besides, availability of nutrients to rice particularly under low nitrogen and low phosphorous levels in the soil are also essential stresses seen in this ecology due to water logging.

2.1.1 Submergence

Under water logging condition, no other staple crop than rice can survive. This unique ability in rice is attributed to its ability to elongate rapidly with onset of water stagnation. But the submergence at early-stage lead to poor crop establishment, submergence at tillering stage suppresses tillering and increases mortality. Excessive water logging for longer time leads to accumulation of toxic substances and crop faces iron toxicity and sulphide injury. The major landmark in the rice gene discovery is submergence tolerance gene in rice research. The submergence tolerance QTL, which was designated as the *Sub1* QTL on chromosome 9 with 70% of the total phenotypic variance in the population (PI543851/IR40931-26) was identified (Xu and Mackill, 1996; Xu et al., 2006). This QTL *Sub1* was recently introgressed into many popular varieties and many submergences tolerant versions namely Swarna-Sub1, Sambha-Sub1, Cherang-Sub1, Ranjit-Sub1, CR1009-Sub1 etc. were developed (Pradhan et al., 2018). The *Sub1* introgressed varieties tolerate up to 12-14 days of submergence, however, due to weather variability and duration of stagnation of water and flash flood duration, new genes should be identified for flood tolerance for 21 days (3 weeks) or more. Rice plants exhibit very limited elongation during submergence and often show tolerance to complete flooding due to a strategy known as quiescence. But, quiescence under deep transient flash flood is limited to few genotypes only (Fukao and Xiong, 2013). Generally, rice accessions contain *Sub1B* and *Sub1C* genes at the *Sub1* locus. Out of which *Sub1A* was reported to contribute ~70% of submergence tolerance to some *indica* and *aus* rice varieties (Xu et al., 2000).

Snorkel 1 (*SK1*) and Snorkel 2 (*SK2*) allow rice to elongate fast whereas Submergence1A-1 (*Sub1A-1*) allows rice to squeeze elongation for adaptation to water stagnation and flash floods conditions, respectively. Both *SK* genes and *Sub1A* encode ethylene-responsive factor, a specific group of transcription factor related to gibberellin biosynthesis or signal transduction. Several mega rice varieties, which were submergence sensitive were converted to submergence tolerant types through introgression of *Sub1A-1* genes by marker-assisted backcross

breeding approach and released for commercial cultivation in different submergence prone areas of Asia. Rice plants adapt to very deep-water stagnation (> 1 m deep) by elongation of culm for escaping out of water by the action of the gene 'Snorkel' 1 and 2 (Hattori et al., 2009). The ICAR-NRRI, Cuttack has identified a novel germplasm (AC 20431B) which tolerates up to 21 days' submergence. The mapping of the gene responsible for 21 days submerge tolerance is continuing using a recombinant inbred population of Swarna-Sub1 and AC 20431B.

2.1.2 Drought

Drought is a common and major occurrence in rainfed shallow lowland ecology. The recent scenario in climate change indicates occurrences of more number and frequency of drought in the eastern region of the country. To safe guard the future production, the variety should have adaption under adverse situation. Several major quantitative trait loci (QTL) under drought were identified and need to be pyramided together to develop drought-tolerant variety. Three QTL namely *qDTY1.1, qDTY2.1* and *qDTY3.1* are showing yield improvement under drought stress (Dixit et al., 2014). The QTL, *qDTY1.1* shows a consistent effect on grain yield under reproductive stage drought. Further, two large-effect QTL which are influencing grain yield (*qDTY2.1* and *qDTY3.1*) show a contribution of 16.3% and 30.7%, respectively, under drought condition. Another QTL, *qDTY12.1* shows consistent additive effects of 45% detected in the marker interval of RM28048 and RM28166 under reproductive stage drought (Dixit et al., 2014). Another large effect QTL (*qDTY3.2*) shows positive effect on yield under drought stress and which is co-localizes with *Hd9*, a locus which is related to days to flowering. Many promising donor germplasm lines *viz*., N22, CR143-2-2, Bala, Lalnakanda, Dagad Deshi, Moroberekan, Aus276, Vandana, Apo and IR55419-04 are in use for drought improvement programmes. Swarna variety carrying drought and submergence tolerance QTL are released as CR Dhan 801 and CR Dhan 802 (Pradhan et al., 2018).

2.1.3 Nutrient deficiency tolerance

Stagnant flooding happens due to heavy rain, which becomes deep-water flooding or flash flooding in the lowlands. Under deep-water flooding, internode elongation in plants occurs to escape the submergence. However, after the flood subsides, the major issues arise are flood affected plants are prone to lodging and ultimately this leads to death due to the exhaustion of reserved nutrients within few days. Nitrogen is the major nutrient input required in large quantities for better production from lowland rice. It plays an active role in yield directly. The N use efficiency in lowland rice is relatively less, since loss in applied nitrogen through volatilization, leaching and denitrification occurs in the flooded soil. The N recovery efficiency (30 –50%) are also relatively low (Fageria and Baligar, 2011).

Phosphorus is usually less deficient in flooded field than in upland rice since available P is higher in flooded soils. But P deficiency is still extensive in lowland rice. There is a marked difference of P in flooded soil than the upland soils. Mostly, after submergence, concentrations of water-soluble P in soil rise. Marked increases in the availability of native and added P in flooded soils have been noted over those in well-drained soils. A major QTL, *Pup1* on chromosome 12 shows 78.8% of the total phenotypic variance for phosphorus uptake and also associated with tolerance to P deficiency and efficient P uptake in low phosphorus soil (Wissuwa et al., 2002). Therefore, the development of N and P-efficient crop varieties that can grow and yield better with low nitrogen and phosphorous is a key to improve rice production in lowland ecology.

3. PHENOTYPIC TRAITS

3.1 Anaerobic germination

Two major QTL *viz.*, *AG1* (*qAG-9-2)* and *AG2* (*qAG-7-1*) associated with tolerance to anaerobic germination have been reported (Angaji et al., 2010). Effectiveness of these QTL in conferring tolerance to flooding during germination stage was demonstrated by introgressing them in the genetic background of two popular rice varieties *viz.*, PSBRc82 and Ciherang-Sub1 (Mondal et al., 2020). One of the identified QTL, *qAG-9-2* has been fine-mapped to *OsTPP7* gene which encodes trehalose-6-phosphate phosphatise involved in starch mobilization during germination (Kretzschmar et al., 2015). Baltazar et al. (2019) reported four QTL conferring anaerobic germination tolerance from the landrace Karsu 80A. Three of the QTL (*qAG7.1, qAG7.2* and *qAG7.3*) were located on chromosome 7 and one QTL was reported on chromosome 3. Using a RIL population derived from the cross YZX x 02428, (Yang et al., 2019) identified stable QTL and candidate genes associated with increased tolerance to anaerobic germination. (Nishimura et al., 2020) by using CSSLs of Koshihikari in the genetic background of IR64 identified a novel QTL, named as *qACE3.1,* on chromosome 3, for coleoptile elongation under flooded conditions. Genetic architecture of anaerobic germination among the deep-water rice population of Assam was unravelled by genome-wide association studies using 50 K rice genic SNP chip (Rohilla et al., 2020). Two genes *OsXHD1* and *SSXT* explained >20% of the phenotypic variation for coleoptile length under water in anaerobic condition in the population. Recently, genome-wide association analysis (Su et al., 2021) has been employed for identifying candidate genes associated with anaerobic germination in rice. The study reported four candidate genes, namely *OsVP1, OsGA2ox8, OsDi19-1* and *Os06g0548200* associated with the trait.

3.2 Root traits

Deep rooted rice avoids drought stress by utilizing moisture from deep soil. All irrigated rice varieties have surface spreading type root system and mild drought stress also affects growth and production. *Dro1* is negatively regulated by auxin and is involved in cell elongation in the root tip that causes asymmetric root growth and down ward bending of the root in response to gravity. High expression of *Dro1* increases the root growth angle, whereby roots grow in a more downward direction. Introducing *Dro1* into a shallow-rooting rice cultivar by backcrossing enabled the resulting line to avoid drought by increasing deep rooting, which maintained high yield performance under drought conditions relative to the recipient cultivar (Pandit et al., 2020). Root growth angle (RGA) is an important trait that influences the ability of rice to avoid drought stress. *DEEPER ROOTING1* (*Dro1*), which is a major quantitative trait locus for RGA, is responsible for the difference in RGA between the shallow-rooting cultivar IR64 and the deep-rooting cultivar Kinandang Patong (Uga et al., 2013). Natural variation in RGA in rice cultivars carrying functional *Dro1* alleles may be controlled by a few major QTL and by several additional minor QTL (Kitomi et al., 2015).

3.3 Lodging resistance and biomass production

The rice breeders in India have typically depended upon dwarfing genes for improving lodging resistance joined with phenotypic selection for stronger culm. The general insight remained that selection of high culm strength may have negative impact on yield also and the variety becomes low acceptable among the farmers due to reduced feed quality of straw. The lodging resistance can be significantly better in rice without any compromise on grain yield. Researchers were able to develop QTL-NILs by transfer of a genomic region from an Indian landrace Kasalath in the background of *japonica* cultivar Koshihikari which could survive two or more moderate typhoons in a particular year without any reduction in yield (Ishimaru et al., 2008). Breeding higher yield in lowland ecology increased the grain and panicle weight. Rice variety carrying a heavy panicle type lodges due to unpredicted rain, wind etc. and may cause high yield loss. The important QTL/genes responsible for lodging resistance in rice are presented in Table 1. The possibility of effective utilization of lodging resistance in rice while addressing the concern of yield and feed quality in Indian rice breeding programme need to be considered. The prospect of combining high biomass production and lodging resistance was demonstrated through development of a long-culm rice forage cultivar named as 'Leaf Star' which was a low-lignin producing lodging resistant rice cultivar suitable for feed and bioenergy production (Ookawa et al., 2014). Culm strength was increased in the mega variety background, Swarna by transferring *SCM2* QTL through marker-assted backcrossing (Mohapatra et al., 2021).

Table 1. List of QTL/ genes identified to confer lodging resistance in rice

Sl. No	QTL/ Gene	Feature	Population	Reference
1	*bsuc11* (Breaking strength upper culm 11)	Present on the Chromosome 11.Improves Breaking strength ofupper culm through thickeningof cortical tissues ininternode and raising the levelof holocellulose.	Chromosome Segment Substitution Lines(CSSLs) developedfrom Koshihikariand Kasalath	Kashiwagi (2014); Kashiwagi et al.(2016)
2	*prl5* (Pushing resistance lower 5)	Present on the chromosome 5. Improves pushing resistance of lower plant parts (*prl5*) by delaying senescence and increasing carbohydrate re-accumulation in stems.	CSSLs developed from Koshihikari and Kasalath	Kashiwagi and Ishimaru, (2004)
3	*lrt5* (Lodging resistance in a typhoon5)	Plants carrying this QTL circumvent domino effect. Starch content of upper culmin *lrt5-NILs* was 4.8 times compared to recurrent parents	CSSLs developed from Koshihikari and Kasalath	Ishimaru et al. (2008)
4	*SCM2* (Strong Culm 2) QTL/ Aberrant Panicle Organization1 (*APO1*) gene	The gene simultaneously improves section modulus of culm along with grain number Per panicle.	CSSLs derived from Sasanishiki and Habataki	Ookawa et al. (2010)
5	*SCM3* (Strong Culm 3) QTL/ Teosinte Branched1 (*OsTB1*) gene	The gene simultaneously improves section modulus ofculm along with grain number Per panicle. NILs of*SCM2* + *SCM3* further improvesboth traits	Backcross InbredLines (BILs) of Koshihikari and Chugoku117	Yano et al. (2015)

3.4 Seed dormancy and seed vivipary

The adverse effects of climate change may result untimed rain during grain maturity stage of the crop. The availability of moisture to matured grain makes the fresh seed to germinate. The farmers suffer a heavy loss due to this vivipary germination of rice. Under shallow lowland condition, seed dormancy is a

requirement for breeding of cultivars as it avoids pre-harvest sprouting of seeds and reduces the quality and storability of the harvest. The Pre-harvest sprouting of seed mostly happens when seed receive sufficient humidity and temperatures during grain filling and maturation, which allow the seed to germinate. Some of the significant findings have been listed in Table 2.

4. BIOTIC STRESS

Although there are a number of formulations of chemicals available in the market for controlling the diseases and insect pests, but the use of chemicals has also several health hazards and environmental concern. Thus, genetic approach to control the diseases and insect pests is always an environment friendly approach. A number of insect pests and diseases reduce the rain yield of this ecology. The insect pests like stem borer, brown plant hopper, leaf folder, gall midge, cutworm and GLH are predominant in the ecology. Main diseases such as bacterial blight, false smut, tungro and sheath blight reduces yield of lowland ecology. Bacterial leaf blight is a major disease of lowland rice ecology which can reduce crop yield up to 50% (Khush et al., 1989) or even up to 80% (Singh et al., 1977). Using the gene pyramiding approach, improved *indica* rice cultivars with broad spectrum durable BB resistance have been developed by combining different genes (Pradhan et al., 2015, 2016, 2019, 2020). A three resistance genes combination appeared to be the most effective with *Xa21*contributing the largest component of resistance. Gall midge is another rice pest reduces rice yield severely. Till date, 11 gall midge resistant genes have been identified. However, no strong host plant resistance genes are identified for use in stem borer improvement programmes. A total of 38 BPH resistance genes/QTL are reported for brown plant hopper (Kumar et al., 2020). Many pyramiding works on bacterial blight has also been performed in the country in various superior backgrounds (Pradhan et al., 2015, 2016, 2019, 2020). The pyramiding work need to strengthen further using multiple biotic stress tolerance genes.

5. GRAIN YIELD

Genomic regions, directly or indirectly contributing to grain yield have been found to be distributed throughout the rice genome and across all the 12 chromosomes. The most important of these genes/QTL, in terms of their practical application in plant breeding, is enlisted in Table 3. As overall grain yield of rice from a crop field is a very complex trait for direct improvement, efforts have been taken worldwide for utilizing the genes and QTL for its component traits. *Gn1a*, a major QTL, has been cloned and characterized for grain number per panicle. The functional analysis shows that *Gn1a* encoded cytokinin oxidase/dehydrogenase (Ashikari et al., 2005). Ideal plant architecture1 (*IPA1*) changes rice plant

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

Sl. No.	Genes/QTL	Population	Reference
1	One QTL (*Sdr1*) for seed dormancy was identified which was very tightly linked to another QTL for heading date (*Hd8*)	BILs from Nipponbare and Kasalath	Takeuchi et al. (2003)
2	Four major QTL and several minor QTL were detected. *qSD-3* on chromosome 3 was most important.	Doubled haploid from *indica* x *japonica*	Guo et al. (2004)
3	Three QTL (*Sdr6*, *Sdr9* and *Sdr10*) were detected	CSSLs of Nona Bokra and Koshihikari	Marzougui et al. (2012)
4	Four putative QTL detected. *qSD-6.1*most effective among those.	BIL of Nipponbare x Kasalath	Sasaki et al. (2013)
5	Eight additive effect QTL for seed dormancy detected. Best combination for pyramiding also suggested.	RILs of *japonica* x *indica*	Wang et al. (2014)
6	Several QTL were mapped at 4, 5 and 6 weeks after heading in the same population	RILs of *japonica* x *indica*	Cheng et al. (2014)
7	Sixteen and 38 loci significantly associated with dormancy in freshly harvested seeds and after ripened seeds. There were three common QTL among them.	Association mapping in global accessions of rice	Magwa et al. (2016)
8	Six major additive effect consistent QTL (*qSD3-2*, *qSD4-1*, *qSD7-1*, *qSD7-2*, *qSD7-3* and *qSD11-2*) with contributions of more than or equal to 30 percent. Validated over seven cropping seasons	Single segment substitution lines of one indica rice with several donors	Zhou et al. (2017)
9	Ten SNPs were identified which significantly affect pre-harvest sprouting	Resequencing of multiple accessions.	Lee et al. (2017)

Sl. No.	Genes/QTL	Population	Reference
10	Identified the QTL (*qSd-1-1*) and the underlying gene (*OsDOG1L-3*) associated with seed dormancy	A NIL of N22 with the *qSd-1-1* allele of Nanjing 35	Wang et al. (2020)
11	A major QTL (*qDOM3.1)* for seed dormancy was identified and a candidate gene (*LOC_Os03g01540*) involved in ABA signalling pathway was identified.	CSSL of Nipponbare in the background of Zhenshan 97	Yuan et al. (2020)
12	Four major QTL (*qSD3.1, qSD3.2, qSD5.2* and *qSD11.2*) for seed dormancy	CSSL and BIL populations derived from Nipponbare and 9311	Zhang et al. (2020)

architecture and enhances rice grain yield. This IPA1 encodes OsSPL14 (Jiao et al., 2010).

The submergence tolerance property of FR13A was first reported by NRRI and shared with other institutes, which was subsequently mapped as *Sub1* and used in rice breeding. The ICAR-NRRI has developed pyramided lines with *Xa21*, *xa13* and *xa5* resistance genes in the backgrounds of Swarna, Jalmagna, IR 64, Lalat and Tapaswini. The Institute also collaborated with IRRI for release of Swarna-Sub1 through SVRC, Odisha. Under 'QTL to variety' project, the Institute has stacked three abiotic stress tolerance genes *viz.*, *Sub1*+ *qDTY1.1*+*qDTY2.1* in the background of Swarna variety. Also, under IRRI-NRRI collaborative project, Swarna variety containing *Sub1*, *qDTY1.1*, *qDTY2.1* and *qDTY3.1* QTL have been released for submergence and drought affected areas of Andhra Pradesh, Telangana, Odisha, Uttar Pradesh and West Bengal states of the country. Bacterial blight resistance genes pyramided line of Swarna has been released as CR Dhan 800 by SVRC, Odisha. Transfer of *Sub1* QTL into the popular lowland varieties like Gayatri, Sarala, Varshadhan, Pooja and Pratikshya through marker-assisted backcross breeding was performed and the Sub-l carrying popular varieties are under advanced stages of national testing. Closely linked markers have been identified for gall midge resistance gene *Gm4* and QTL for BPH resistance (Mohanty et al., 2017). The Institute has released several varieties namely Pooja, Sarala, CR Dhan 500, CR Dhan 401, Gayatri, Savitri, Dharitri, Swarna-Sub1, CR Dhan 800, CR Dhan 801, CR Dhan 802, CR Dhan 505, CR Dhan 506 and CR Dhan 508 for cultivation under shallow lowland ecosystem (Table 4).

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

Sl. No.	Yield Genes/ QTL	Traits	Chr.	Donor line/ variety	Gene function	Reference
1	*Gn1a*	Grain number	1	Habataki	Cytokinin Oxidase/ Dehydrogenase2	Ashikari et al. (2005)
2	*SPL16*	Panicle branching	8	ST12	Squamosa Promoter binding protein like-16	Wang et al. (2012)
3	*SCM2*	Culm diameter	6	Habataki	F-box containing protein	Ookawa et al. (2010)
4	*Ghd7*	Delayed heading date and increased plant height and yield	7	Minghui63	CCT domain protein	Xue et al. (2008)
5	*GS5*	Grain length	5	Zonhhua11	Serine carboxy peptidase	Li et al. (2011)
6	*GW5*	Grain weight	5	Nipponbare	Unknown protein	Weng et al. (2008)
7	*TGW6*	Test grain weight	6	Kasalath	IAA glucose hydrolase	Ishimaru et al. (2013)
8	*DEP1*	Erect panicle, high grain number	9	Shenung265	Phosphatidyl ethanol-amine binding protein	Huang et al. (2009)
9	*SPIKE1*	Grain number/ panicle	4	IR68522-10-2-2	Polar auxin protein	Fujita et al. (2013)
10	*GW2*	Grain weight	2	Oochikara	Ring-type E3 ligase protein phosphatase	Song et al. (2007)
11	*GS3*	Grain length	3	Minghui63	Trans membrane protein	Fan et al. (2006)
12	*GS2*	Grain size, grain yield	2	Baodali	Growth-regulating factor 4	Zhang et al. (2013); Hu et al. (2015)
13	*qTGW3*	Grain size, grain weight	3	CW23	Glycogen Synthase Kinase	Hu et al. (2018)
14	*GW6a*	Grain weight, grain yield, biomass	6	Kasalath	GCN5-related N-acetyl transferase-like histone acetyl transferase 1	Song et al. (2015)
15	*GS9*	Grain shape, quality	9	Qingluzan11	Transcriptional activator	Zhao et al. (2018)
16	*WTG1*	Grain size, grain shape	8	Zhonghu-ajing mutant	Otubain-like protease with deubiquitination activity	Huang et al. (2017)

Table 4. Rice varieties developed by the institute for shallow lowland ecosystem

Sl. No	Variety name	Year of release	Maturity duration (t/ha)	Potential yield	Grain type*	Special feature**	Recommended States
1	Anamika	1979	145	4.5	LB	-	Tamil Nadu
2	Ramakrishna	1980	130	4.0	MS	BLB, GM, tolerant to water logging, iron toxicity	Odisha
3	Samalei	1980	150	4.5	LS	GM, BL	Odisha
4	Savitri	1982	155	5.5	SB	BL, BB, MR	Andhra Pradesh, Odisha, Tamil Nadu
5	Utkalprabha	1983	155	4.0	MS	MR or field tolerance to major pest and diseases	Odisha
6	Dharitri	1988	150	5.0	SB	BI, BB, SB, GM	Odisha
7	Padmini	1988	145	4.0	MS	BB	Odisha
8	Moti	1988	145	5.0	LS	BL, RTV, GLH	Odisha
9	CR 1014	1988	160	3.5-4.0	MS	MR to Sh. B. MRor field tolerance to all pest and diseases	Odisha
10	Gayatri	1988	160	4.0-6.0	SB	MR to Sh. B. MR or field tolerance to all pest and diseases	Odisha, West Bengal, Bihar
11	Kalashree	1988	160	4.0	MS	Tolerant to Blast & GM	Odisha
12	Panidhan	1988	180	3.5-4.0	MS	Tolerant to Blast & GM	Odisha
13	Tulasi	1988	170	3.5-4.0	MS	Field tolerance to major pests and diseases	Odisha
14	CR 1002	1992	145	4.5-5.0	SB	ShB, GLH	West Bengal, Odisha, Bihar

Sl. No	Variety name	Year of release	Maturity duration (t/ha)	Potential yield	Grain type*	Special feature**	Recommended States
15	Seema	1992	150	4.5-5.0	MS	Blast, BPH, GM, tolerant to water logging	Odisha
16	Pooja	2000	150	5.0	MS	BL	Odisha, Madhya Pradesh, West Bengal
17	Sarala	2000	150	4.75	MS	Intermediate non- lodging, Photosensitive	Odisha
18	Durga	2000	155	4.3	MB	Resistant to RTD and suitable for late planting	Odisha
19	Varshadhan	2006	160	3.5 -4.0	LB	Non lodging and suitable for water logging situation	Odisha
20	Hanseswari (CR Dhan 70)	2008	150	3.0	SB	MR-Blast,ShB, Tol-False Smut, RTV	Odisha
21	Swarna -Sub1	2009	143	7.0	MS	Tolerant to complete submergence for two weeks	Odisha, Uttar Pradesh
22	Reeta (CR Dhan 401)	2010	150	7.5	MS	BL, NB, BS, ShB, ShR, SB, LF	Odisha,West Bengal, Tamil Nadu, Andhra Pradesh
23	Nua Chinikamini	2010	145-150	3.5	SB	RTV, SB, RGM, NB, ShR	Odisha
24	CR Dhan 501	2010	152	3.4	LB	R-Neck blast	UP, Assam
25	CR Dhan 500	2011	160	7.2	MS	MR to LB, NB, GM, SB, LF	Odisha, Uttar Pradesh
26	Sumit	2012	145-150	7.5	LB	LB, SB, LF	Odisha
27	Poorna Bhog	2012	140-145	5.2	LS	NB, GM, ShR, SB	Odisha
28	CR Dhan 503 (Jalamani)	2012	160	4.9	MS	MR-LF, GLH, LB, NB, BS, G, SB	Odisha

Sl. No	Variety name	Year of release	Maturity duration (t/ha)	Potential yield	Grain type*	Special feature**	Recommended States
29	CR Dhan 502 (Jayanti dhan)	2012	160	5.3	MS	MR-LB, NB, ShB, ShR, RTV, GM, SB, LF, RT, WM	Odisha
30	CR Dhan 407	2014	150	5.8	LB	MR to leaf blast, neck blast	Odisha, West Bengal
31	CR Dhan 505	2014	162	7.1	MS	Submergence tolerance	Odisha, Assam
32	CR Dhan 408 (Chakaakhi)	2014	165	7.5	LB	MR-LB, NB, BB, ShR, SB, LF, WBPH	Odisha
33	CR Dhan 508	2016	160	8.5	LB	MR-NB, BS, ShR, SBR, LF, WM	Odisha, Assam, West Bengal
34	Pradhan Dhan (CR Dhan 409)	2016	160	7.8	LS	MR-LB, NB, ShR, SBR, LF	Odisha
35	CR Dhan 507	2016	165	6.8	MB	MR-NB, BS, ShR, ShB, SBR, WM, LF	Odisha
36	CR Dhan 910	2016	140-145	5.6	MS	MR-BL, NBl, ShR, RTV, SB, LF, WBPH	Odisha
37	CR Dhan 909	2017	140	6.5	MS	Tolerant to LB, NB, ShR, RTD	Uttar Pradesh, Bihar, Assam, Maharashtra
38	CR Dhan 801	2017	140	7.8	MS	For sub-mergence and drought prone areas	Uttar Pradesh, Bihar, Odisha, West Bengal
39	CR Dhan 506	2017	165	4.2	LB	Leaf blast, neck blast, brown spot, sheath blight, sheath rot, tungro virus, stem borer, leaf folder and whorl maggot	Assam, Andhra Pradesh, Karnataka

Sl. No	Variety name	Year of release	Maturity duration (t/ha)	Potential yield	Grain type*	Special feature**	Recommended States
40	CR Dhan 802 (Subhas)	2019	142	6.5	SB	Submergence and drought tolerant, it is resistant to stem borer, leaf folder, plant hopper and case worm while moderately resistant to bacterial blight, sheath rot and rice tungro virus	Bihar, Madhya Pradesh
41	CR Dhan 510	2019	160	4.0-5.2	SB	MR to blast, BB, SBR & LF	WB and Odisha
42	CR Dhan 511	2018	160	4.7	SB	Moderately resistant to leaf blast, neck blast and bacterial blight diseases, stem borer, white backed plant hopper and leaf folder	WB, Odisha, Andhra Pradesh
43	CR Dhan 410 (Mahamani)	2020	160-165	7.0	LS	Moderately resistant to stem borer, leaf folder, neck blast, bacterial blight, sheath rot and brown spot	Odisha

6. STRATEGIES FOR GENETIC IMPROVEMENT FOR CULTIVARS IN LOWLAND RICE

Efforts are being made to reduce the yield limiting factors of lowland ecology of rice. However, the changing climatic pattern and severity of stresses poses more challenges for rice cultivation. During last few decades, high yield potential

varieties have been developed, however, the yield productivity challenges still continued. The climate resilient varieties for lowland ecosystem are one of the keys to improve the productivity and for stabilization of the production from the harsh ecologies. Most of the varieties of this ecology are photosensitive in nature, higher maturity duration, hence one generation only can be advanced in a year. Thus, it reduces the number of generation advancement per unit of time. Presently, breeding cycle is much longer in lowland ecology compared to other irrigated and upland ecology, so the rate of genetic gain also expected to be very low. Three kinds of approaches can be adopted together to expedite the process of development of high yielding stable varieties viz.

6.1 Gene stacking

Stacking of multiple functional stress tolerance gene(s)/QTL to make climate smart varieties. Effects of the QTL/ gene(s) need to be segregated out first and then combination effects need to be studied so that proper pyramiding as well as stacking combinations to be identified. The yield QTL under drought, N-use efficiency at low N level, kneeing ability, bacterial blight resistance genes, gall midge resistance genes, seed dormancy and yield enhancing QTL need to be stacked to make them highly resilient to the climate change. Pyramiding of genes/ QTL for anaerobic germination ability, tolerance to excess water submergence during germination and vegetative stages for getting successful crop at direct seeded rainfed lowland areas.

6.2 Reducing breeding cycle

Speed breeding employing the field RGA need to be used for reducing the breeding cycle for rapid development of thousands of lines at a time. This would help in increasing the efficacy in selection of appropriate genotypes with higher yield and leads to rapid genetic gain.

6.3 Identification of suitable donor and functional gene for the trait improvement

Development of molecular markers for the submergence tolerance particularly for three weeks (21 days) will be highly useful for improvement of submergence tolerance. Multiple stress tolerance lines including biotic stresses, anaerobic germination, lodging resistance, nitrogen and phosphorus use efficiency are required in the lowland breeding materials.

Moreover, genetic enhancement of the breeding materials against toxic and other problematic soils needs more attention in future. Study on development of host plant resistance for the emerging diseases and insect pests need to be taken up in an environment friendly manner. In future, production of rice needs

to be increased from lesser land area. For this purpose, the efforts are being focused towards increasing per plant yield through higher grain number per panicle and grain weight. This will lead to long, heavy panicle making the plant more prone to lodging. At this point to avoid yield loss due to higher panicle weight, lodging resistance gene also need to be stacked. On an average, the yield loss measured 35-85% in case of semi-dwarf varieties. Efforts to increase yield potential of the lowland rice can be expedited in a holistic manner to get a quantum jump in yield from lowland areas of eastern India.

7. FUTURE THRUST/CONCLUSION

India has the largest area under rice cultivation, but is only the second largest producer (117.94 Mt during 2019-20) behind China due to low average yield of 2.7t/ha. The low national average productivity can be attributed mainly to the fact that a substantial area under rice cultivation in India belongs to the rainfed low land ecology which is prone to highly unpredictable and extreme situations of abiotic stresses. The average productivity of Eastern and North Eastern India where the rainfed low land ecology is concentrated, is 2.44 t/h and 2.21 t/ha, respectively which is much less than the national average. Hence to improve the overall rice productivity of the country, major emphasis needs to be given for the rainfed lowland ecology. The breeding efforts should focus on the development of varieties having multiple abiotic stress tolerance and yielding ability under uncertain environmental conditions. Due to the erratic rainfall, lodging and viviparous germination has become a major threat to sustained rice production during the recent years. Hence research efforts are required to develop varieties with strong culm and having minimum of 7 days of dormancy. At the policy level, a 'Rainfed Lowland Rice Shuttle Breeding Network' need to be constituted by bringing together research stations working on rainfed lowland ecology, so that the breeding lines could be evaluated more effectively in their target environments. This will lead to the development of varieties with wider adaptability, high resilience, stable yield, and high productivity under the unpredictable abiotic stresses prevalent in the rainfed low land rice ecologies in the Eastern and North Eastern India.

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Irrigated Rice Breeding

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SUMMARY

Around three fourth of total rice production, comes from irrigated rice which is about 55% of total rice land area in World and India as well. Rice production in India has made tremendous progress over the years. However, it is facing unprecedented challenges of environmental degradation and climate change in recent years. At the current level of per capita availability rice production is required to go up by 40 and 70%, by 2030 and 2050, hence productivity has to enhance further. Genetic enhancement is one of the most potential approaches of higher productivity. Early work on rice basically focused on improving native varieties of highly heterogeneous native farmers varieties with resistance to different biotic and abiotic stress situations. Systematic efforts by national and international agencies with collaborative approach focused on improvement of yield and other traits mostly through breeding and selection utilizing diverse germplasm. Breakthrough in yield potential was achieved through modification of plant type, first by introducing dwarfing gene '*sd1*'from the dwarf plant type Dee-Geo-Woo-Gen, which ushered green revolution in mid-sixties. Subsequent development focussed more on stability of the varieties incorporating more disease, pest resistance as well as earliness, however yield remains static. Then research on New Plant Type for improvement of dry matter and harvest index for enhancing yield potential was targeted. Similarly, hybrid rice also played a significant role for upgrading the productivity pattern. In the meantime many QTL for yield and yield attributing traits and genes for major diseases and pests have been identified which paves the way for molecular breeding. Similarly, transgenic approach of yield enhancement was also tried with limited success. The present strategy 'the modernization of breeding initiative includes both modification of classical breeding along with utilization modern breeding schemes for incorporating high precision tools and genomic selection on the basis of necessary demands. To support this pre-breeding of wild ancestors and physiological manipulation are also necessary without compromising diversity.

Keywords: Irrigated, unprecedented, genetic enhancement, heterogeneous, yield, rice

1. INTRODUCTION

India occupies the largest rice area in the globe *i.e.*, about 43 million ha and is the second highest producer with about 120 million tonnes of milled rice followed by China. It is the staple food for more than 65% of Indian population contributing approximately 40% to the total food grain production, thereby, has a pivotal role in the food and livelihood security of people. It is the predominant dietary energy source and economically, socially and culturally considered important for consumers in many Asian countries, where 90% of total rice is produced and consumed (Siddiq and Vemireddy, 2021). However, the rice productivity is only 2.56 tonnes $ha^{-1}$of milled rice in contrast to global average of 3.01 tonnes ha^{-1}.Considering the escalating population growth rate of 1.63%, the supply projection the target of rice production is estimated to be 197.4 m. tonnes by 2050 (Pathak et al., 2020).Similarly, the average global rice yield increase is 1.0% per year which is far short of<"2.4% (non-compounding) and therefore, it is required to double the rice production by 2050. At the current growth rates, only <"42%production increases in riceis possible by 2050 (Ray, 2013).

Globally, 75% of total rice production comes from irrigated rice which is about 55% of total rice land area (Peng, 2008). Similarly, in India the importance of irrigated rice is evident, as 70% of rice production is achieved from about 50% of rice area (Singh, 2009). The irrigated rice area is about 26 million hectares, which accounts about 60.0 % of the total area under rice in the country Irrigated rice is grown in bunded fields and irrigation is the main source of water in the dry season while supplemental irrigation is provided in wet season in addition to rainfall. The major rice producing states in the country are West Bengal, Uttar Pradesh, Punjab, Odisha, Andhra Pradesh, Bihar and Chhattisgarh.

Over the years, area under rice has increased about 1.5 times, while there is fivefold increase in production enabling the country toachieve self-sufficiency and surplus for export. Rice production in India has made tremendous progress over the years. However, it is facing unprecedented challenges of environmental degradation and climate change in recent years (Pradhan et al., 2018; Pradhan et al., 2019). At the current level of per capita availability rice production is required to go up by 40 and 70%, from 116 to 145 and 190 million tonnes of milled rice respectively by 2030 and 2050. We would be therefore, require to add annually not less than 3.0-3.5 million tonnes of milled rice to sustain the present level of self-sufficiency and surplus in rice (Siddiq, 2013). It is therefore, a challenging task to achieve such a huge targeted production levels in the next few decades as increase in productivity has to come from the declining and degrading natural resource bases, growing labour and energy shortages and threats of climate change. Further, the shortage of ideal land, irrigation water, inputs and genetic resources may aggravate the problems.(Pathak et al., 2019; Dash et al., 2018).

2. CHALLENGES OF IRRIGATED RICE

a. Yield enhancement: The average yield of irrigated rice is substantially high; as compared to rice harvested from other ecologies, but substantially lower than the potential yields. It needs sustained effort for further yield enhancement.

b. Depleting ground water: Rice cultivation requires a large amount of water. More than 5,000 liters of water is required for producing one kg of rice. Inefficient irrigation water management coupled with the over dependence of paddy (because of obvious reasons) has serious impact on water crisis in India.

c. Major problems encountered in this production system are yield instability due to environmental degradation which might be rooted to unbalanced nutrient use. There is declining of nutrient use efficiency also aggravates this problem. There are also reports of micronutrient deficiency in many places including indo-gangetic plains in the country due to continuous rice wheat cropping system.

d. Over-mining of nutrients and/or faulty irrigation caused salinity/alkalinity; delayed planting due to uncertainty of canal water release in command areas; imbalanced fertilizer/ nutrient use, sub-optimal plant population; widespread micronutrient (Zn) deficiency and high incidence of pests and diseases (due to general wet condition) constitute the major constraints in rice production (Siddiq, 2000). Salinity and deficiency of P, Ca, S, Zn; low fertilizer use; occasional submergence/moisture stress coupled with lack of ideal varieties seriously affect productivity in the eastern and western zones.

However, in this chapter, we focused mostly on increasing the genetic potential for productivity enhancement and associated issues in irrigated rice.

3. PIONEERING WORKS ON RICE IMPROVEMENT

Early work was basically focused by improving native varieties by pure line selection of highly heterogeneous native farmers varieties. In India, early rice improvement programme was mainly concentrated on the selection of best plants among the populations from the popular local varieties. Many of the improved varieties like T 141, T 90, T 1242, Latisail, Manoharsali, MTU 15, CO 25 etc. were developed and successfully adapted by the farmers. Some of them were popular for long time. Similarly, varieties were developed with resistance or tolerance to different biotic and abiotic stress situations e.g., FR 13A and FR 43B in India and Kurkaruppan of Sri Lanka for flash flood tolerance (Mohanty and Chaudhury, 1986). As the varieties were selected from landraces and farmers variety, therefore, could sustain well under low management practices along with some stress tolerance, despite low yield . The Central Rice Research Institute (CRRI) was established at Cuttack, India in 1946 by the Govt. of India, and The International Rice Research Institute (IRRI) in 1960 was memorable in the history of rice research

due to their role for further development of leading Indian varieties. CRRI started well organized introduction of Chinese, Japanese, Taiwanese, Indonesian and Russian types of genetic stocks along with methodical screening and evaluation. Establishment of the International Rice Commission (IRC) under the Food and Agriculture Organization (FAO) of the United Nations, in 1949 was a milestone in the advent of collaborative and cooperative rice improvement focusing on yield and other traits mostly through breeding and selection (IRC, 1950). While yield improvement had been the major objective from the early period of rice improvement, other factors responsible for low productivity might be the limitations of the varieties under cultivation *viz*., susceptibility to diseases and insect pests, late maturity, lodging, shattering of grains; and a narrow range of adaptation.

A breeding program with inter–subspecific hybridization programme between short *japonicas* (procured from Japan and other regions of Eastern Asia*)* and *indicas* (tall tropical *indicas)* by International Rice Commission (IRC) during 1950-54 by FAO through a collaborative project in South-East Asian countries, imparted some basic but historic success. The main outcome of the project was release of Malinja and Mahsuri in Malaysia, ADT-27 in Tamil Nadu, India, Norelon -340 in Philippines and Circna in Australia (Parthasarathy, 1972). Although it was viewed as a programme of limited success, the variety Mahsuri happens to be the parent of present day mega varieties such as Swarna (Vasistha/ Mahsuri) and Samba Mahsuri (GEB 24/TN 1// Mahsuri).

In India, breeders 'focused more on selecting long duration varieties for *kharif* season as the largest land area was covered only in wet season. Moreover, the idea was also that longer duration provides better opportunity for higher productivity. However, later on it was proved that there is hardly any correlation between late maturity and high yields. Hence, more efforts were there on breeding photo period insensitive varieties that also can be grown under both the seasons. Early-maturing varieties-of 100 days duration were developed. They were suitable for *boro* and *aus* varieties in *rabi* and *kharif* seasons in India, and in multiple cropping systems. In IRRI, few potential early maturing types were chosen from IR 1561 lines (1R747B2-6 x 1R579-48-1). Several other breeding lines, such as IR579-48-1 (IR8 x Tadukan); IR747B2-6 (Peta/3 x Taichung Native **1)** x TKM-6/2, were used as promising early maturing parental lines for further breeding of early HYVs. These materials possess seedling vigor and moderately high levels of insect and disease resistance, combining multiple traits were important for early maturing as well as high yield (Beachell et al., 1972). Breeders during early 1950s could identify bacterial leaf blight as one of dreaded diseases along with blast and *Helmintho sporium* for irrigated ecosystem.

4. PRODUCTIVE STRATEGIES OF YIELD IMPROVEMENTS

4.1 Classical breeding

4.1.1 Breakthrough in yield potentiality using dwarfing gene

As tall *indicas* were cultivated mostly during 1950s, lodging was one of the major problems, and aggravated with more use of fertilizers. Culm strength of the varieties was usually weak and lodge badly even under standard management practices. Lodging tolerance was urgently required, so short stature and stronger culms were major focal points. Tropical tall *indicas* used to shatter to some extent hence, loss after lodging was difficult to avoid. Continuous efforts lead to incorporation of some degree of lodging tolerance, however, those failed to stand erect under moderate to high doses of fertilizer (Parthasarahy, 1972). Moreover, cultures having lodging resistance were associated with poor tiller and grain yield. Nevertheless, some of the improved varieties in India were incorporated with non-shattering habits *viz.*, S 22, MTU 27, PTB 9, CO12, GEB 24, and CO 25. Similarly, varieties that respond to high levels of fertilization were rare among the *indicas.* Still, some of them with better fertilizer responsiveness were developed *viz.*, MTU 2, MTU 10, CO 20, and S. 601etc.

Further, short statured tropical *japonicas* with stiff straw were focused. The pioneer in this aspect happened to be a high yielding variety, Taichung Native I, was developed from Taichung station in 1956 from cross of Dee-geo-woo-gen x Tsaiyuan-chung. It was also found to be efficient in nitrogen responsiveness, that even up to 100 kg/ha. Dee-geo-woo-gen was a semi-dwarf amply tillered variety while Tsai-yuan-chung was endowed with tallness and disease resistance. Similar kind of short statured genotypes identified were, l-geo-tze and Taichung Sen 2, Kaohsiung Sen 2 and Ai-chueh-chien. Apart from that, C4-63 was developed at Philippines (Escuro et al., 1969) and IR5 at IRRI that represented varieties of intermediate height in which the reduction in plant height was visible. In the meantime, a significant finding drawn the attention of rice workers that, only when the tall, vigorous, tropical *indica* genotypes are dwarfed, they do exhibit high yield potential. The tropical *indica* varieties were enriched with superior agronomic traits and had clear-cut advantage over semi-dwarf *japonica* and U.S. varieties largely because of their superior vegetative vigour, high tillering, and high leaf area index. However, because of their obvious problem of lodging, shattering and lack of fertilizer responsiveness, dwarf varieties like TN1 was extensively preferred by farmers and breeders in India in mid 1960s despite its disease susceptibility. Similarly, TN1 and I-geo-tze were used in the breeding programs of IRRI along with the national programmes in Sri Lanka, India, Thailand, Republic of Korea, and Surinam (Huang et al., 1972). IRRI scientists choosen Dee-geo-woo-gen, the culture with semi-dwarf plant stature as a parent,

and hybridized with Peta, a vigorous tall variety from Indonesia leading to one of the highly successful variety in the history, *i.e.,* IR8 was accepted far and wide because of its very high yield potential. Subsequently many improved varieties were developed utilizing IR 8 as an important source of dwarfing gene.

The severe lodging and mutual shading of the tall, tropical *indica* prevented the use of large amounts of nitrogen fertilizer which are essential for high grain yields. With the incorporation of dwarfing genes, tropical tall *indica* cultures were dwarfed and lodging was reduced along with communal shading that resulted in plant type amenable for high nitrogen fertilizer. Fortunately, it responded with high grain yield even when leaf area index is relatively high. The tropical *indica* varieties were enriched with clear-cut advantage over semi-dwarf *japonica* and U.S. varieties largely because of their superior vegetative vigor, high tillering, and high leaf area index.

Despite the impact of varieties such as TN1, Mahsuri, and ADT 27, rice farmers in tropical and subtropical Asia were still growing numerous unimproved or semi-improved varieties during early 1960s, without the use of modern agricultural knowledge. Because of their inherent disadvantage of tallness, lack of culm strength, late maturity, photoperiod insensitiveness, lack of broad spectrum resistance to disease and pests, they could be adapted to limited geographical area. Average productivity in such varieties particularly in tropical rice-growing countries were abysmally low to the tune of 1-2 t/ha. However, the population growth of these countries were distinctly higher than food production. This trend bound to result in severe food shortage leading to extensive famines in the late 1970s (Paddock and Paddock, 1967; Borgstrom, 1972). However, introduction of such HYV varieties and further improvement by national programmes, in rice and wheat along with advanced management practices have enhanced the productivity 2-3 times higher than that of the traditional varieties. Wide-scale adoption of these varieties and proper agronomic practices has led to major increases in food production. World rice production increased 82% from 257 million tonnes in 1965 to 468 million tonnes in 1985. World wheat production increased 89% from 267 million tonnes in 1965 to 506 million tonnes in 1985. Gradually, with the further advancement of science, instead of food scarcity we have now sufficient food. However, further production required to feed the ever increasing population.

However, large scale dependence on only few semi dwarf sources (dominated by Dee-geo-woo-gen), due to their short stature and nitrogen responsiveness the chance of epiphytotic condition (for diseases and insects) could not be ruled out. Hence, development of a broad range of varieties with different background was emphasized seriously with improved plant type. The rice breeders

were suggested to use local varieties in crosses with such dwarf gene derived varieties having improved plant type. It was emphasized that more divergence may break the linkage with any undesirable traits, or even susceptibility to disease or insects that are closely linked with dwarfing genes. Hence, efforts in this direction lead to several HYVs of superior productivity, *viz*., Jaya (TN1 x T-141) & Padma (T141 x TN1) in India, and BG 90-2 in Sri Lanka from *japonica / indica* hybridization programme. Similarly, interspecific hybridization in Korea, resulted Tongil-type rice varieties during 1971, with a yield enhancement up to 30% compared to *japonica* varieties. These varieties had a great role in securing self-sufficiency and food security in Korea and led to 'Korean Green Revolution (Chung and Heu, 1980). These genotypes were endowed with medium-long and erect leaves, thick leaf sheaths and culms, dwarf statured plant along with long panicles with lodging resistance. The same inter racial hybridization of *indica/ japonica* hybridization by Japanese breeders in 1982 led to the varieties with bulky panicles along with high grain numbers per panicle such as Akenohoshi and Akichikara and termed as super-high-yielding cultivars (Wang et al., 1997). The green revolution gene '*sd1*'in the dwarf plant type Dee-Geo-Woo- Gen was discovered subsequently led the path of precise breeding in plant type concept. Sufficient genetic diversity was encashed using *indica and japonica* in transgressive segregants along with other economically useful traits required for higher productivity potential eliminating the necessary bottlenecks prevailing thereof.

Subsequent breeding initiatives were towards yield increment did not result much success towards productivity enhancement rather than sustenance of high yield or yield stability with incorporation of resistance against diseases and insect pests, because of its obvious menace in yield reduction. Another major issue was reduction of growth duration. Beachell, Khush and the IRRI team could develop varieties *viz*., IR28 and IR30 in1974 and IR36 in 1976 maturing in 110 days. Furthermore, reduction in growth duration was accomplished with development of varieties like IR50 (105 days) and IR58 (100 days) enhanced the productivity based on per day yield. During the selection of these varieties, one thing was emphasized seriously not to compromise on yield. Therefore, short duration genotypes that are comparable for grain yield to the medium duration varieties were selected. The factor pivotal for success of these early varieties was selection of segregants with fast growth for vegetative stage (Khush, 1987). Because of quick early growth, these were more competitive against weeds and hence cost of weed control reduced. As these varieties mature 20-25 days early, less utilization of water resources, pesticide requirements are also minimized too; making it cost effective.

During that period, variety IR64 was developed which happened to be highly successful, and could accumulate the similar total dry matter in 85-90

days as the medium duration varieties do in 115-120 days. To add upon, the harvest index of these short duration varieties was slightly superior to the medium duration varieties. Further, Khush and IRRI team could develop IR72, with yield potential on par with IR8 but have shorter maturity duration and added with resistance to multiple disease and pests. The important observation was when productivity potential was compared; it showed 5-10% higher than IR8 on per day basis. In addition to it, superior grain quality was also supplemented. However, overall yield potential of semi-dwarf *indica* inbreds remained static at this stage too.

Rice improvement programs in several tropical countries developed short-duration varieties with comparable productivity potential as that of medium or late duration. Ratna, Cauvery, and TKM9 in India, BG34-7 and BG367-7 in Sri Lanka, and Chandina in Bangladesh are well-known among them. These concerted efforts led to increased productivity, self-sufficiency, sustainability and even surpluses in several countries of Southeast and South Asia. There was an appreciable improvement of production in rough rice to 513 M tonnes in 1996 from 240 M tonnes in 1956 to, an appreciation of over 114% (2.1 t/ha in 1966 to 3.8 t/ha in 1996). Correspondingly, in India, there is vertical expansion of rice production to 81.7 M tonnes from 30.4 M tonnes, a magnification of 168% comparable to horizontal expansion of 23.3% (35.2 mha to 43.4 mha) (Annonymous, 2000; Pingali and Hossain M, 1998). This increase in production could outpace the population growth of 82% leading to food security in the continent.

4.1.2. Plant type concept

The trend of overall yield increment had a set back further. In this context, physiologists started analyzing the basis of yield vigour in rice. The semi-dwarf rice varieties in the 1960s were the most striking example of a successful improvement in plant type. It was postulated that the yield plateau of semi-dwarf *indica* rice varieties might be the result of the following reasons.

a. Plant type, with several unproductive tillers, confined sink size and even many of them are prone to lodging, which results in yield stagnation.

b. There was superfluous leaf area causing mutual shading and a reduction in canopy photosynthesis and sink size.

There is possibility of breaking of yield plateau by necessary remodeling of the contributing parameters (Dingkuhn et al., 1991). In this context, it was conceptualized to modify the ideotype of irrigated and other ecologies in the early 1990s.

Several approaches were there for breaking yield ceiling in irrigated ecosystem, and the new plant type (NPT) was one of the potential approach conceptualized at IRRI by Dr. G.S. Khush. It hypothesized augmentation of total dry matter and HI leading boost to the productivity. HI was supposed to be strengthened by higher sink capacity along with short sturdy stem, erect, short, narrow, thick and dark green leaves. These cultures also believed to be greater responsiveness to applied N and higher yield potential. The target was to develop a plant type with yield increment up to 30-50% than the available best variety in dry season (Khush, 1995).

Donors with large panicle, thick stem, short stature and low tillering *bulu* genotypes or *javanica* (*Tropical japonica*) type germplasm from Indonesia, Malaysia, Thailand, etc. were selected and hybridization was done to get the First generation NPTs with heavy panicle, shy tillering and lodging resistance traits. The idea was to develop a plant type with the following features (Peng et al., 2008):

a) Low tillering capacity with 3-4 panicles /plant (direct-seeded)/8-10 panicles (transplanted) with a view to eliminate unproductive tillers.

b) Heavy panicle with 200-250 grains per panicle.

c) 90-100 cm tall.

d) Thick and sturdy stems.

e) Erect, thick leaves with dark green colour.

f) Vigorous root system.

g) Multiple disease and insect resistance.

h) 110-130 days growth duration.

i) Harvest index of 0.6.

j) 13-15 t/ha yield potential.

The performance of the 1st generation NPTs was not satisfactory because of fewer tillers along with low biomass production and spikelet sterility. However, the promising genotypes were crossed with elite and popular *indicas* to develop second generation NPTs. With necessary trait specific augmentation, few lines could out yield IR 72 (Peng, 2004) and IR72967-12-2-3 produced 10.16 t /ha, higher to *indica* check PSBRc52. Similarly, few were released in Philippines and China (Peng, 2008). However, the tiller number was increased and the grain number reduced to 150 (maximum). Hence, many of the expected features could not be materialized including yield.

In china, with the influence of IRRI's NPT programme and super high yielding hybrid rice combination, a super' hybrid rice concept was initiated in

1998 by Prof. L. P. Yuan. The approach was to combine an ideotype with the use of inter-sub-specific heterosis (Yuan, 2003). The plant type was little bit modified to accommodate the traits *viz.*, tall erect leaf canopy for the first three leaves, moderate tillering capacity, large panicle and high HI (Peng, 2008). Here high biomass was perceived through little raised plant height along with longer and wider top three leaves. The panicle was projected to be inside the canopy. It should be droopy and the tip of the panicle should be at a height of around 60 cm from the soil surface (Peng, 2008) (Fig. 1).

a) Top three leave blades should be long, erect so that it will have a larger leaf area but also can receive light on both sides: Flag-leaf length of 50 and 55 cm for the 2nd and 3rd leaves respectively. All three leaves are above panicle height.

b) Should remain erect until maturity. Leaf angles of the flag, 2nd, and 3rd leaves are ~ 5^0, 10^0, and 20^0, respectively.

c) Narrow and V-shape leaves (2 cm leaf width) with stiffness.

d) Thick leaves (specific leaf weight of top three leaves = 55 g/m^2) (thick leaves have delayed senescence and higher photosynthetic efficiency).

e) Leaf area index (LAI) of top three leaves is about 6.0.

f) Plant height of at least 100 cm (from soil surface to unbent plant tip) and panicle height of 60-70 cm (from soil surface to the top of panicles with panicles in natural position) at maturity.

g) Heavy (5 g per panicle) and droopy panicles at maturity.

h) Moderate tillering capacity (270–300 panicles /m^2).

i) Theoretical yield potential is 15 ton/ha.

Few Chinese hybrids *viz.*, Xieyou 9308 and Liangyoupeijiu could reportedly achieve grain yield to the tune of 12.23 t/ ha and 12.11 t/ha respectively with some desirable features (28% and 17.5% higher than hybrid checks).

4.2 Modern breeding strategy

Considering the inbred rice varieties, the yield stagnation still remains an issue and is a matter to be worried considering the genetic gain for productivity to be at a sluggish rate of approximately 1% per year (Peng, 2000). Hence, it was seriously re-evaluated by IRRI scientists and tried to get into the core of the breeding system and was found that the basic methodology that controls the variety development was pedigree method and around 78% breeders use it as their primary scheme (Lenaerts, 2018) and that to a single location up to F_6 or F_7. In this context, it was conceptualised to make it more efficient as that of private

sector, *i.e.*, market driven, product oriented pipeline in order to have greater genetic gain comparable to a manufacturing unit (Rayes, 2014). The breeding methodology was dissected in the following components (Collard et al., 2019).

4.2.1 Demand-led Breeding, understanding market trend and client need

The breeding objectives were restructured and planned keeping in view the future market trend and consumer's need based on systematic market study on various stakeholder's (farmers, millers, dealers, distributers, exporters, retailers and consumers) preference. The traits were decided by the end users rather than breeders. Based on the realistic data necessary product profiles were designed based on a large amount of data collected from the consumers in the form of survey and group discussion with farmers. Analysis of data bring forth the guidance to the breeders in fixing priorities and fine tuning the quality traits along with desired stress tolerance and nutrient use efficiency as desired by the farmers for replacing the existing variety. Product profiles were supposed to the key guideline even for designing the hybridization programme and selection strategy. Overall, it was also important for allocation of resources in real term.

4.2.2 Modern Breeding Schemes (Population Breeding)

The rice breeding program traditionally relied on conventional pedigree breeding scheme for development of variety. The method involves single plant selection in each generation emphasizing selection for major morphological traits / genes. Another limitation is confinement of selection to a single location for 6-7 generation, limiting the scope of GxE interaction. It was replaced by a more efficient population breeding scheme, modified SSD, where advanced F_5 derived lines are planted in the field for seed increase rather than in F7 stage. This facilitates the yield testing in small plots more swiftly. The population breeding scheme allows minor alleles of the quantitative traits *viz.*, grain yield. The focus of modified breeding scheme is to increase genetic gain for productivity by adopting "Population breeding" in combination of accelerated product development *viz.*, Rapid generation advance.

4.2.2.1 Speed Breeding: Accelerated product development

Traditionally in pedigree breeding approach it could accommodate 1-2 generations per year. It offers only limited number of fixed lines for yield testing. In contrary, many "Accelerated product development technologies" have emerged from both public and private sector are available and provide opportunities to take three to four generations per year. Rapid Generation Advance (RGA) enables growing of breeding population in modified environment in order to shorten the life cycle, allowing us to take 3-4 generations per year. RGA is a proven to be feasible option, could save huge resources in terms of land, labour and time for developing genetically fixed lines. RGA, Field-RGA and Field nursery strategies are usually

techniques for speedy development of genetically fixed lines in large numbers per cross (Collard, 2017). Population breeding approach was taken in combination with RGA for quick and efficient breeding.

4.2.2.2 Genomic selection & Precision breeding

DNA markers are being utilised in breeding programme for screening of traits where linked markers or gene specific markers are available. This increases the efficiency of screening of traits where markers are identified. This is largely utilised in breeding for imparting resistance to biotic and abiotic stress along with quality. Efforts have been made to optimise marker applications in IRRI TRB irrigated breeding program. Regarding trait-specific marker genotyping, this was accomplished by replacing SSR, CAPS and InDel with SNP markers facilitating high throughput fluorescence-based platforms, using the Fluidigm platform, and KASP (Kompetitive Allele Specific PCR) system (Semagn, 2014). In order to make it highly user friendly, a minimal 10 SNP quality control set with optimized allele frequencies was developed to distinguish any two *indica* lines by at least one polymorphic SNP. This quality control set found widespread use in parental verification before hybridization, hybridity verification post crossing, and genotype variation throughout the breeding and seed production pipelines. MAS was utilised for forward breeding scheme so that less efficient genotypes are eliminated at an early stage. Here the basic principle was emphasized to utilise MAS for forward breeding of important traits (with marker availability) at an early stage in the breeding programme for elimination of inferior breeding lines. This MAS could also be highly useful in RGA to eliminate poor lines during the process of line fixation and effective reduction of population size. The RGA breeding method is highly suitable for integrating MAS because screening can be performed during line fixation and be used to reduce population sizes. Our scheme was also designed to over-lay genomic selection in order to further increase the rate of genetic gain (Heffner, 2010).

Genomic selection (GS) (also called "genome-wide prediction") has been suitably utilised for quantitative traits such as grain yield (Jannink, 2010). In this case, selection was practiced based on whole-genome marker information called 'genomic estimated breeding values' (GEBVs) which is a solitary score for each trait, rather than on individual genes or QTL (Nakaya, 2012) was utilised for choosing highly effective parents for precision of parental selection for effective genetic gain in transgressive segregants.

4.3 Increased selection pressure and smart data collection

There was intense selection pressure for grain yield (*i.e.*, grain yield per se measured in plots). Selection was also exercised while advancing the segregating

generations particularly to fixed lines ($F_{5:6}$). Rejection was more stringent to the entries particularly for height, flowering duration, and grain type. Unless the stipulated criteria are met in early generation, further advancement or retesting was not allowed. Further, the number of genotypes at the observational yield trial stage (OYT) were enhanced from 600–800 to about 1500 entries with plot sizes of 4 or 5 m^2 with sufficient precaution for correct experimental design and collection of experimental data.

Smart data collection components were introduced to minimize human error, precision and efficiency in breeding activities significantly. Electronic data collection encompasses bar-coding, electronic data capture, use of PB apps and hand-hold devices, printers, scanners etc. Similarly, a partially replicated (p-rep) design was employed in PYT, which was reported to be highly efficient for multi-location trials (Moehring et al., 2014). Similarly, for data analysis, a two-stage model was selected. In stage 1, fixed effects was considered whereas, spatially adjusted best linear unbiased estimates (BLUEs) for each line at each location was taken into account in stage 2, where genotypes were considered random effects and environmental main effects and genotype × environment interaction variance components are calculated. The resulting best linear unbiased predictors (BLUPs) for individual genotype are subsequently utilised for selection (Smith, 2005).

One innovative strategy was the introduction of multi-location testing at the OYT and preliminary yield trial (PYT) stages rather than post PYT phase (AYT) at least in 3-4 locations (Collard,2019)

4.4 Research at National level and Contribution of NRRI

After the introduction of green revolution varieties, *viz*., TN1, IR 8, IR 36 and IR 64, the *tropical indica* germplasm were successfully utilized as one of the parent to introduce potential alleles in the transgressive segregants along with dwarfing genes successfully. Further, elite lines or varieties were intercrossed to develop number of potential varieties in irrigated ecology. However, the *indica* are still large reservoir of favorable alleles. Similarly, *Tropical japonica*, *aus* and aromatic derivatives were also utilized in the existing breeding programme to develop more than 1300 high yielding varieties in India. Some of the highly popular HYV in this ecology were having high yield and field resistance are IR64, MTU1001, MTU1010, MTU1153, MTU1156, Shatabdi, Sahbhagidhan, Naveen, Pratikshya, Kalachampa, CR Dhan 310, CR Dhan 601, Mahamaya, Rajeswari, Zinco Rice MS, Lalat , Lalat MAS, CO-51, DRR-44, IR -64 Drt1, Telengana Sona, Jagtial Mashuri, JGL-18047, KNM-118 and Gontra Bidhan-1 etc. Out of those, 149 varieties have been developed by ICAR-NRRI, Cuttack for different ecologies.

Table 1. List of popular irrigated varieties released by ICAR-NRRI, Cuttack

Sl. No.	Variety	Year of release	Released by CVRC/SVRC	Duration	Grain Type	Reaction to diseases & pests and special characters
1	Khitish	1982	West Bengal	120	LS	Suitable for dry season, 5.5 t/ha
2	Tapaswini	1996	Orissa	135	MS	MS; Res. to BLB, WBPH, 5 t/ha
3	Shatabdi	2000	West Bengal	110	LS	MR-BB, SH.B, Sh. Rot, Suitable for dry season, 3.7 t/ha
4	Abhishek	2006	CVRC (UP, Bihar, Jharkhand, Assam)	125	SB	SB; Semi dwarf (95-110 cm), R-BL,; MR- BS,GM ; 4.5-5.0 t/ha
5	Chandrama	2006	Assam	130(Irrig) 170 (Boro)	SB	SB;R-Blast, MR-BLB, RTV, Sh Bl, SB, BPH, WBPH, GM, 15 d dormancy
6	Geetanjali	2006	Orissa	130	LS)	Aromatic, semi-dwarf; R-NB, MR-GM,4.5 t/ha
7	Naveen	2006	Orissa, Tripura, WB	120	MB	105 cm; R-BL; 5.0-5.5 t/ha
8	Rajalaxmi (Hybrid)	2006	CVRC (Orissa, Assam)	135	LS	MR- SB, 5.85 t/ha
9	Ajay (Hybrid)	2006	Orissa	130	LS	MR-BL, BLB,SB, BPH, WPH, GM, 6.07 t/ha
10	Satya Krishna (CR Dhan10)	2008	Orissa	135	LS	R- NBl, ShR; MR-SB, GM, WM, 5.2 t/ha
11	Chandan (CR Borodhan 2)	2008	CVRC (Orissa, Assam)	125 (Irrig) 145 (Boro)	MS	MR-SB, BPH, BL, BLB, ShBl, 6.1 t/ha
12	Phalguni	2010	Orissa	117	LS	105 cm , R- BL, MR-ShR, RTD, BS, ShBl, YSB, WBPH, BPH, GLH, R- GM, LF, 4.0-4.5t/ha in *Kharif* 5.0 – 6.0 t/ha in *Rabi*
13	CR Dhan 601	2010	CVRC (Orissa, WB and Assam)	125(Kharif) 160 (Boro)	MS	90cm, R- BL, RTV; MR-BS, ShR, SB, GLH, LF, Tolerant to Cold; 5.6 t/ha
14	Improved Lalat	2012	Odisha	130	LS	R- BLB, gall midge and MR-stem borer, 4.5 to5.0t/ha

Sl. No.	Variety	Year of release	Released by CVRC/SVRC	Duration	Grain Type	Reaction to diseases & pests and special characters
15	CR Dhan 300	2013	CVRC (Maharashtra, Gujarat,Odisha, Bihar)	140	LS	R- LF, MR-LB, NBL, ShR and SB, 5.0-5.5 t/ha
16	CR Dhan 303	2014	CVRC (MP, UP, Odisha)	125	SB	115 cm height, MR- BL, NBL, ShR and RTV, 5.0 t/ha
17	CR Dhan 305	2014	CVRC (Jharkhand, Maharashtra and Andhra Pradesh)	125	SB	LS; MR- leaf blast, BPH and WBPH, 4.8 t/ha
18	CR Dhan 304	2014	CVRC (Odisha and West Bengal)	130	SB	R- GM , 5.0 t/ha
19	CR Dhan 307(Maudamani)	2014	Odisha	135	SB	110 cm, MR-LB, BS ShR, SB, LF 110cm, 4.8 t/ha
20	CR Dhan 310 (IET 24780)	2015	Odisha, Madhya Pradesh & Uttar Pradesh	125	MS	110cm, High protein grain (10.5%), 5.0 t/ha
21	CR Dhan 311 (Mukul)	2016	Odisha	120-126	LB	115cm,Tol- BL, GD, BS, RTD, BLB, MR-GM, SB, Protein 10.1%, Zn-20 ppm, 5.54 t/ha

Grain: LS - Long Slender MB -Medium Bold, MS -Medium Slender, SB-Short Bold, MB-Medium Bold; Resistance: BL-Blast, NBL- Neck blast, BLB - Bacterial Leaf Blight, BPH - Brown Plant Hopper, BS -Brown Spot, GB -Gundhi Bug, GM -Gall Midge, GLH - Green Leaf Hopper LF -Leaf Folder RTV -Rice Tungro Virus, SB -Stem Borer Sh.B - Sheath Blight, Sh.R -Sheath Rot, WBPH - White Back Plant Hopper, SB -Stem Borer

Coming to irrigated ecosystem, 52 varieties have been developed by the institute till date. Some of the highly popular varieties have been enlisted below to improve the production.

4.5 Ideotype research at NRRI

The IRRI NPTs in the late segregating stage were obtained and selection was exercised in a climate conducive to eastern India to establish superior lines (NPTs). Some of these NPTs were found with good yield potentiality, and even some of those showed the productivity of more than 9.0 t ha^{-1} during dry season 2012 (Donde et al., 2020). However, there was lack of stability. In this context, the

concept of NPT was revisited and new generation rice (NGR) was suggested. It was conceptualized to have more dry matter by more of leaf area materialized through moderately high tiller number, moderately long and wide leaves and high harvest index. It is supposed to produce productivity potential of at least 20% higher than the popular mega varieties or best check vis-à-vis resistance to abiotic and biotic stress along with acceptable grain quality. Moreover, it should have stable yield performance even under light stress. Hence, with an objective of development of indigenous, very high yielding NGR, the NPT lines along with standard popular *indica* check varieties and *Tropical japonica* lines were selected for their *perse* performance and combining ability. Different sub species of *O. sativa* was studied at molecular level and five distinct groups, corresponding to *indica, aus*, aromatic, *Temperate japonica,* and *Tropical japonica* rice were identified. Nuclear and chloroplast data supported a closer evolutionary relationship between the *indica* and the *aus* and among the tropical *japonica*, temperate *japonica*, and aromatic groups (Garris *et al*., 2005). Divergent genotypes in the backdrop of high yield NPTs *viz*., IR 73963-86-1-5-2-2, IR 72967-12-2-3 and IR 73907-753-2-3 with good GCA, were hybridized with a set of promising *Tropical japonica*, *indica* and aromatic lines with potential yield and yield attributing traits to obtain some high performing NGRs. Even some *indica* cultures with untapped yield potential were intercrossed to develop high performing NGRs.

Varieties CR 3856-44-22-2-1-9 (CR Dhan 316) and CR 4113-3-2-1 (CR Dhan 314) were found to be most potential with heavy panicles (4-6 g/panicle), high grain number (200-300) with good quality (medium slender grains/ long bold grains, 22.0-24 g/1000 grains), moderate tillering (6-9), plant height (115-120cm), moderately long and wide semi erect top three leaves, thick culm etc. Such variety have been reported with yield potentiality of 8-10 t/ha. Similarly, the variety namely, CR Dhan 307 a transgressive segregant from *indica* intercross has been released in 2015 with above described NGR characters along with 'V" shaped erect leaf has been reported with more than 10 t/ha yield potential in farmers' field (Dash et al., 2018; Pandit et al., 2021). However, these are also associated with some bottlenecks *viz*., disease pest resistance and minor quality issues which need improvement for yield stability. Similarly, augmentation for little more tillering and spikelet fertility could further improve its yield potential as well.

There were many recombinants with heavy panicles (5.0 g to 15.0g) however, maximum of them could not be finally converted into superior population performance, hence, could not out yield the check parents. This was may be either due to non-uniform panicle type in all tillers, or may be due to less tillers or due to high spikelet sterility. Again, these NGRs with heavy panicles were found to be more prone to biotic stresses. In this context, promising

recombinants or elite lines could be intercrossed based on their phenotypic performance or GEBV for trait specific supplementation along with incorporation of disease/pest resistance is expected to make these NGRs more stable along with higher productive.

5. HYBRID RICE

Heterosis is the superiority of F_1s over either parent, apprehends ample hybrid-vigour in crop plants, helped the farming communities and fascinated geneticists for breeding excellence over centuries (Verma et al., 2018). The hybrid breeding is one of the greatest discoveries in the history of rice farming. Suitable allelic combination has made yield advantage in hybrid than HYVs (Rout et al., 2020). Hybrid rice (HR) is a practically feasible and readily adoptable to enhance productivity up to 15-30% (Rout et al., 2020). Given to its remunerative yield, several rice hybrids are commercialized in over 40 countries which create huge business opportunities. Hybrid rice was first time commercially realized during seventies of 20th century in China which contributed enormously towards Chinese food security and livelihood (Yuan et al. 2017). In India, HR technology in project mode was launched as 'Promotion of research and development efforts on hybrids in selected crops' by ICAR during 1989 at 12 centres. Subsequently, first hybrid APRH-1 was released by APRRI, Maruteru during 1994 and country became 2nd after China to commercialize HR.

At the beginning, this program was technically supported by the IRRI, Philippines and Food and Agriculture Organization (FAO), Rome and financially supported by United Nations Development Programme (UNDP), Mahyco Research Foundation, World Bank funded National Agricultural Technology Project (NATP) and IRRI/ADB etc. Government of India has supported scaling up and promotion of HR through various schemes. This technology is further sustained by ICAR through platform research project 'ICAR - Consortium research platform on Hybrid Crops Hybrid technology for higher productivity in selected field and horticultural crops, at the 09 research institutions in the country. Indo-ASEAN agency is also funded substantially for exploitation of more heterotic inter-subspecific genetic pool for further breeding excellence in HR. International Rice Research Institute, Philippines has provided all requisite genetic materials, CMS *viz.*, V 20A, Yar Ai Zhao A, Wu10A, MS 577A, Pankhari, 203A, V 41A, Er-Jiu nanA; respective B-lines, and 9 other maintainers; and 13 good restorers (Anonymous, 1981-82). So far, country has commercialized a total of 127 rice hybrids (38 from public and 89 from private sector) suitable for diverse ecologies (115-150 days) which covers ~3.5 mha of country's total rice acreage (Varietal Improvement, Progress report, IIRR, 2020). Besides, country has also developed >100 stable CMS and respective maintainers indigenously under diversified genetic

and cytoplasmic background. The promising CMS lines like CRMS 8A, CRMS 31A, CRMS 32A, CRMS 53A, CRMS 54A, CRMS 56A, PMS10A, DR8A, PMS 17A, APMS 6A, PUSA 5A, RTN 12A, PUSA6A, etc. are being utilized in HR breeding programs in India and abroad. Remarkably, a medium duration CMS, CRMS 32A having seedling stage cold tolerant is developed in the Kalinga-I cytoplasm. The hybrids released in India are adaptable to tropical and subtropical ecologies, having unambiguous specificity towards habitat, sustainability and consumer preferences. Early duration hybrids, DRRH-2, Pant Sankar Dhan-1, Pant Sankar Dhan-3and KJTRH-4 are found suitable for rain-fed upland ecology. Medium duration hybrids, DRRH-28, Pant Sankar Dhan-3, KRH-2, HRI-148, JRH-8, PHB-71, and Rajalaxmi are adaptable under salinity; and Suruchi, PHB-71, JKRH-2000, CRHR-5, DRRH-2, DRRH-44 and Rajalaxmi are suitable for alkalinity stress. *Boro* season which is known to be more productive is hybridized with rice hybrids, Rajalaxmi, CRHR-32, NPH 924-1, PA 6444, Sahyadri, KRH 2, RRX-113 and RRX-336. Improved hybrids like BS 6444G, Arize Prima, Rajalaxmi, Ajay, CR Dhan 701, PRH 10 and Arize AZ 8433 DT are found suitable for productivity enhancement in eastern region of the country. Hybrids like 25P25, 27P31, CRHR 105, CRHR 106 and CRHR 150 are heat tolerant in nature, hence, suitable for yield enhancement under heat stress. The hybrids, JKRH 401, US 382, US 312, Indam 200-17 and DRRH3 are highly N use efficient, thus, are appropriate for the soil which is poor for N content. Moreover, hybrids, RH 1531, Arize Tej, CRHR 105, CRHR 106 and PNPH 24 are mid-early maturing varieties suitable for water scarcity situation. The coastal and shallow-lowland areas which is sharing ~ 32% of the total rice area but remains low productive, can be benefited by adopting late maturity HR varieties, CRHR 32, Arize Dhani, CRHR 34, CRHR 102, CRHR 103 and Sahyadri5.

The ICAR-National Rice Research Institute started breeding on HR during 1979 (Anonymous, 1981-82). Institute has commercialized three hybrids, Ajay (125-130 days), Rajalaxmi (125-130 days; 168 days under *boro*) and CR Dhan 701 (140-145 days) covering about ~0.174 mha rice area covering the states of Odisha, West Bengal, Bihar, Assam, Gujarat and Tripura during *kharif,* 2019.These are commercialized under public-private partnership mode and licensed to 19 seed agencies. Besides, the Institute has developed a total of 57 stable CMS lines (WA, Kalinga-I and *O. perennis*etc. MS cytoplasm), respective maintainers and >100 good restorers. Among the CMS lines, Annada A (WA), Pusa 33A (WA), Sarasa A, Manipuri A (WA), Kiran A (WA), Moti A (WA), Deepa A (WA), Krishna A *(O. perennis),* Krishna A (Kalinga I), Padmini A, Mirai (Kalinga I), PS92A and Sahbhagidhan A etc. are prominent to be utilized for hybrid development. The CMS, CRMS31A (WA) and CRMS32A (Kalinga-I) are substantially utilized in varietal development in India. The medium late duration CMS, CRMS24A,

CRMS40A and CRMS 56A (>45% outcrossing) are suitable for development of late duration hybrids; and short duration CMS lines namely, CRMS8A, CRMS51A, CRMS52A, CRMS53A, CRMS 54A are useful for hybridization for rainfed-upland hybrid development (Verma et al., 2018).

Biotechnological intervention in HR breeding has made remarkable progress in enhancing breeding efficacy and varietal development. It helped HR in various ways like mining desirable traits and their incorporation, molecular characterization etc. Varietal identity/purity of hybrids and parents is mandatory to assure the proprietorship (IPR issue) and pure seeds to the stakeholders, performed through grow-out-test (GOT) which is very expensive and time consuming. Genomic tools in this regard established as impartial and appropriate alternate for GOT. Several co-dominant DNA-based markers are developed and utilized for genetic purity analysis in hybrids/parents (Behera et al., 2012; Verma et al., 2017). Besides, genomics-assisted selection/MABB/genome editing tools is providing strong utensils for improvement of trait of interest in crop plants. Rice hybrids, Rajalaxmi, Ajay (Das et al., 2016), PRH 10, BS 6444G (Basavaraj et al., 2009), and parents of CR Dhan 701 (Das et al., 2016) etc. has been improved for bacterial blight (BB) resistance. The blast and BB resistance genes are also successfully stacked in popular CMS, Pusa 6A (Singh et al., 2015). Besides, sustainability of hybrids under abiotic stress like salinity and submergence are improved by deploying tolerant QTL in CMS, CRMS 31A and CRMS 32A. The quality of HR which is major concern is improved by stacking QTL/genes for quality traits in parents, Zhenshan 97A (Zhou et al., 2003), Xieyou57 (Ni et al., 2011). The yield related QTL *yld1.1* and *yld2.1*. were introgressed in restorer 'Ce64' from *O. rufipogon*. (Duan et al., 2013).Hybrid sterility (HS) is common annoyance threatening the inter-subspecific hybrids (5-10% more heterosis), caused by the genes at *Sb*, *Sc*, *Sd* and *Se* (Guo et al., 2016) and the gene at *S5* locus. It is successfully addressed through genome editing tool 'CRISPR/Cas9' (Shen et al., 2017). Genomic selection (GS) has made harnessing heterosis more precise and accurate (Lu and Xu, 2010).

HR technology has supplemented food security substantially in rice growing countries in temperate zones. However, it could not make substantial dent in tropics where owing to low photon availability during wet season, yielding ability of HR varieties remains meagre. Under the changing climatic and agriculture scenario rice hybrid is likely to face stiff competition to sustain in future. Despite having great potential to enhance production and productivity, it has not been adopted on large scale as was expected. This is due to several constraints like lack of acceptability of hybrids across the country due to region specific grain quality requirement. Moderate (15–20%) yield advantage in hybrids is not economically

very attractive, needs strategic breeding intervention to increase the extent of heterosis further. Lower market price offered for the hybrid rice produce by millers/traders is acting as a deterrent for many farmers to take up hybrid rice cultivation. Higher seed cost is another restrain for large scale adoption and hence there is a need to enhance the seed yield in hybrid rice seed production plots. Efforts for creating awareness and for technology transfer were inadequate in initial stages. Involvement of public sector seed corporations in large scale seed production has been less than expected. Hybrids rice for aerobic/upland, *boro* season and long duration hybrids for shallow lowland conditions need to be developed. Most of the constraints mentioned above are being addressed with right earnestness through the on-going research projects and transfer of technology efforts.

6. MOLECULAR BREEDING FOR IMPROVING YIELD, AGRO-MORPHOLOGICAL AND YIELD-RELATED TRAITS

Several agro-morphological and yield-related traits like plant height, tiller number, grain number, grain weight, grain yield, etc are mainly controlled by many QTL. Thousands of QTL/genes have been reported for these traits (www.gramene.org; TARO). However, few QTL/genes for these traits have been used in MAS programs in order to have better plant architecture and higher yield, which is one of the major objectives of the breeding programs. Several QTL/genes have been cloned, for example, heading date (*Hd1* to *Hd17, HBF1, HAPL1, DTH3, HDR1, Ehd4, OsMADS3, OsMDAS6, OsMADS18, OsMADS22, qDTH-5, qDTH8, Ghd7*, etc); plant height (*sd1, d10, d11, htd1, Dwarf 3 (d3), Dwarf 10 (D10), Dwarf 14 (D14), Dwarf 17 (D17),* etc); tiller number (*MOC1, Dwarf1 (HTD1), Dwarf27 (d27), OsIAA6, PAY1*, etc); grain number (*Gn1a, GNP1, DEP1, IPA1/OsSPL14, OsSPL16, GAD1, An-1, DTH7/Ghd7.1, Ghd7, DTH8/Ghd8,* etc); grain weight and size (*GW2, TGW3, qSW5/GW5, TGW6, GW8, GS3, GS5, GS9, qGL3, GL2, GL3.1, GL7, GLW7, GW6a/OsglHAT1, OsAGSW1,* etc); and panicle branching (*Gn1a, IPA1/OsSPL14, OsSPL17, LAX1, DEP1, EP2)/DEP2, DEP3, EP3/LP, FZP, OsOTUB1, IPA1, IPI1, OsNAC2,* etc) (Li et al., 2021; Zhong et al., 2021)

Ashikari et al. (2005) developed NILs for *Gn1a* in the background of Nipponbare from donor Habataki and observed an increase in grain number. Zhang et al. (2008) transferred *qSPP7* and *qGPP* QTL into Zhenshan 97 background from HR5. The NILs showed an increase of 50% spikelet per panicle and 80% variation in plant height and heading date. Ando et al. (2008) developed NILs by transferring *qSBN1* and *qPBN6* from Sasanishiki, a *japonica* cultivar into *indica* cultivar Habatak. The spikelet per panicle was reported to have 30% higher than Habataki. Negrão et al. (2010) introduced the *sd1* gene into two traditional *japonica* varieties, IR36 and Allorio (IRGC 284), and observed a reduction in height. Imai et al. (2013) introgressed six yield QTL *yld1.1*, *yld2.1*, *yld3.2*, *yld6.1*, *yld8.1*, and

Table 2. List of important QTL/genes identified for agro-morphological and yield-related traits in rice*d1*

QTL/ gene name	RAP-ID	Traits	Chrom#	Encodedproduct	References
Hd1	Os06g0275000	Heading date, photoperiod sensitivity	6	Protein with a zinc finger	Endo-Higashi and Izawa et al.(2011); Brambilla and Fornara (2013)
Ghd7	Os07g0261200	Height and heading date	7	CCT domain protein	Xing and Zhang et al. (2010); Miura et al. (2011)
Ghd8/DTH8, qHD5	Os08g0174500	Heading date	8	OsHAP3subunitofa CCAAT-box binding protein	Miura et al. (2011); Yan et al. (2011); Brambilla and Fornara (2013)
Ehd1	Os10g0463400	Early heading date	1	B-type response regulator	Endo-Higashi and Izawa et al. (2011)
Hd5	Os08t0174500	Heading dateShort-day promotion, long-day repression	8	B-type response regulator putative HAP3 subunit of the CCAAT box-binding transcription factor	Schmidt et al. (2014)
Sd1	Os01t0883800	Semi dwarf, green revolution gen	1	Similar to GA C20oxidase2	Sasaki et al. (2002)
D14	Os03g0203200	Regulation of shoot branching, high-tilleringdwarf mutant	3	Alpha/beta-fold hydrolase superfamily(probably involved in strigolactone signaling)	Minakuchi et al. (2010)
DLT	Os06g0127800	Dwarf and low tillering	6	GRAS family protein (involved in brassinosteroid response)	Tong et al. (2012)

QTL/ gene name	RAP-ID	Traits	Chrom#	Encodedproduct	References
D17/ HTD1	Os04g0550600	high-tillering dwarf1	4	Carotenoid-cleaving dioxygenase (CCD)(involved in strigolactone biosynthesis)	Minakuchi et al. (2010)
D10, D15, D16	Os01g0746400	Regulation of tillering, dwarf	1	CCD8 (involved in-strigolactone biosynthesis)	Minakuchi et al. (2010); Yano et al. (2015)
D3	Os06g0154200	Bunketsuwaito tillering dwarf3	6	F-box leucine-rich repeat protein (involved in SL signaling)	Minakuchi et al. (2010)
D27	Os11g0587000	Bunketsuto tillering dwarf27	11	â-carotene isomerase, Iron-containing protein, (involved in strigolactone biosynthesis)	Minakuchi et al. (2010)
SRS5	Os11g0247300	Twisted dwarf1	11	Alpha-tubulin protein, cell elongation	Segami et al. (2012); Huang et al. (2013)
MOC1(GNP6)	Os06g0610350	Control of tillering, monoculm1	6	GRAS family nuclear protein	Xing and Zhang (2010), Zhang et al. (2020)
Dwarf 1 (HTD1)	Os04t0550600	High tillering dwarf	4	Carotenoid cleavage dioxygenase	Hong et al.(2005)
OsIAA6	Os01t0741900	Drought tolerance, control of tiller outgrowth	1	Auxin-responsive protein	Jung et al. (2015)
PAY1	Os08t0407200	Plant architecture and grain yield	8	Auxin polar transport and distribution	Zhao et al. (2015)
LAX1	Os01g0831000	Lax panicle1, regulation of axillary meristem formation	1	Basichelix–loop–helix, (bHLH) transcription factor	Xing and Zhanget al. (2010)

QTL/ gene name	RAP-ID	Traits	Chrom#	Encodedproduct	References
LAX2	Os04g0396500	Lax panicle2, regulation of axillary meristem formation	4	Nuclear protein with a plant- specific conserved domain	Tabuchi et al. (2011)
TLD1	Os11g0528700	Increased number of tillers, enlarged leaf angles, and dwarfism 1	11	Indole-3-aceticacid(IAA)-amido synthetase	Zhang et al. (2009)
GS3	Os03g0407400	Long kernel3	3	Transmembrane protein	Miura et al. (2011); Mao et al. (2010)
GS5	Os05g0158500	Positive regulator of grain size	5	Serinecarboxy peptidase	Li et al. (2011); Huang et al. (2013)
GW2	Os02g0244100	Regulation of grain width and weight	2	RING-typeE3ubiquitin ligase	Miura et al. (2011)
GW8	Os08g0531600	Control of grain size, shape and quality	8	Transcription factor, Squamosa promoter-binding-like protein 16	Wang et al. (2012)
qSW5/ GW5	Os05g0187500	Regulation of grain width and weight	5	IQ calmodulin-binding motif family protein	Miura et al. (2011)
PGL1	Os03g0171300	Positive regulation of grain length and weight	3	A typical basichelix–loop–helix (bHLH) protein	Heang and Sassa (2012)
HGW	Os06g0160400	Heading and grain weight	6	Ubiquitin-associated (UBA), domain protein, Proline-rich cell wall protein-like.	Li et al. (2012)
SRS3	Os05g0154700	Regulation of grain length and plant height, small and round seed	5	Novelkinesin13protein	Kitagawa et al. (2010)

QTL/ gene name	RAP-ID	Traits	Chrom#	Encodedproduct	References
FLO2	Os04g0645100	Grain size and starch quality, floury endosperm (grain fertility)	4	Novel protein with three repeat of tetratricopeptide repeat (TPR) motif	Wang et al. (2010)
GSN1	Os05t0115800	Negative regulator of grain size, positive regulator of grain number	5	Mitogen-activated protein kinase (MAPK) phosphatase,	Guo et al. (2018)
Gn1a / OsCKX2	Os01g0197700	Grain number	1	Cytokininoxidase/ dehydrogenase	Ashikari et al. (2005); Sakamoto and Matsuka (2008)
EP3/LP	Os02g0260200	Panicle architecture, erect panicle, larger panicle	2	Kelchrepeat-containingF-boxprotein	Piao et al. (2009); Li et al. (2013)
Ngl f - 1 (*OsARG*)	Os04g0106300	Panicle development and grain production, narrow grain and low fertility	4	Arginine hydrolysis enzyme	Ma et al. (2012)
APO1	Os06g0665400	Inflorescence form, Lodging resistance and grain yield	6	F-box protein	Ikeda-Kawakatsu et al. (2012)
APO2/ RFL	Os04g0598300	Aberrant panicle, short and solid culm, control of inflorescence, flower development	4	Plant-specific transcription factor (LFY homolog)	Ikeda-Kawakatsu et al. (2012)
WFP /	Os08g0509600	Ideal panicle architecture	8	Squamosa promoter-binding like transcription activator	Miura et al. (2010); Jiao et al. (2010); Luo et al. (2012)

QTL/ gene name	RAP-ID	Traits	Chrom#	Encodedproduct	References
IPA1, IPA1/ OsSPL14	Os03g0753100	Inflorescence and spikelet development	3	MADS-box protein, OsMADS34	Kobayash et al. (2010)
PAP2	Os07g0669500	Frizzy panicle, determination of panicle branching and spikelet formation	7	ERF transcription factor	Xing and Zhanget al. (2010); Katoand, Horibata (2012)
FZP	Os09g0441900	Panicle architecture, panicle erectness	9	Phosphatidylethanolamine-binding protein (PEBP) like domain protein	Xing and Zhanget al. (2010); Zhou et al. (2009); Taguchi-Shiobara et al. (2011)
DEP1/ DN1/ qPE9-1	Os07g0616000	Dense and erect panicle2, regulation of panicle erectness, panicle length and grain size	7	Novel plant-specific protein	Zhu et al. (2010); Li et al.(2010); Abe et al. (2010)
EP2/ DEP2/ SRS1	Os08g0162100	Aberrant spikelet and panicle1,regulation of meristem fate	8	Transcriptional co-repressor	Yoshida et al. (2012)
ASP1	Os03g0706500	Fine culm1, negative regulator of lateral branching	3	TCP-domain transcription	Sakamoto et al. (2008); Xing and Zhanget al. (2010); Minakuchi et al. (2010); Guo et al. (2013); Yano et al. (2015)
OsTB1/ FC1	Os04g0460600	Regulation of shoot	4	Similar to H0219H12.10 protein. NAC transcription facto	Jiang et al. (2018)

QTL/ gene name	RAP-ID	Traits	Chrom#	Encodedproduct	References
NAC (*OsNAC2*)	Os09t0456100	branching, plant height and flowering time	9	Remorin_C-containing protein	Hong et al. (2016)
LP1	Os03g0123300	Long panicle	3	Activator of the anaphase promoting complex/ cyclosome (APC/C) complex	Lin et al. (2012); Xu et al. (2012)
TAD1/TE	Os04g0413500	Tillering and dwarf 1, regulation of postembryonic shoot branching and tillering	4		
GIF1	Os06g0660200	Regulation of endosperm development(grain fertility)	6	Cell wall invertase	She et al. (2010)
OsPIN2		Control of canopy architecture		Auxin efflux transporter	Chen et al. (2012)

yld9.1 into Jefferson's background from *O. rufipogon*. The NILs carrying the *yld2.1* and *yld6.1* showed an increased yield of 27.7% and 26.1%, respectively. Luo et al. (2013) introgressed*qSPP5* and *qTGW5* into the Hwayeongbyeo background from *Oryza rufipogon*. There is an increase in 10-15% spikelet found in the NILs. Wang et al. (2015) developed NILs carrying QTL, *GW7*, *OsSPL16,* and *GS3*. They observed that NILs having both the QTL (*GW7* and *GS3*) increased the grain yield. *GW7* when interacting with *OsSPL16* increases the grain yield and improves grain quality. Kim et al. (2015) developed NILs with two QTL *tgw2* and *gw8.1*. They found that the QTL *tgw2* increased the grain weight up to 22.2%, *gw8.1* up to 7.8%, where both the QTL in a single background increased the grain weight up to 32.4%. Wang et al. (2017) introgressed two yield genes for secondary branch number (*SBN*) and spikelet per panicle (*SPP*) into a *japonica* cultivar Tainan 13 from the donor IR65598-112-2.They found that the BC_2F_5 lines had an increase in *SPP* and *SBN* by 83.2% and 61%, respectively. Singh et al. (2018) introgressed*qGn4.1* for grain numbers per panicle into 12 rice mega varieties. All the varieties showed an increase in grain number. Kim et al. (2018) introgressed *Gn1a* and *OsSPL14* into the elite *indica* cultivar background. They reported that the *Gn1a* does not affect yield in *indica* background, whereas *OsSPL14* increased the grain number per panicle by 10.6-59.3%. Pandit et al. (2021) enhanced yield in the irrigated variety, Maudamani by introducing yield component QTL, *GW5*. Five target genes/ QTL including yield component QTL, *OsSPL14* and *SCM2* were transferred into the variety, Swarna by adopting marker-assisted backcross breeding approach. Most of the popular mega varieties like Swarna, Samba Mahsuri, MTU1010, etc are found to be partial or incomplete restorers, and hence cannot be utilized for the hybrid breeding programs in order to boost yield. Ponnuswamy et al. (2020) transferred fertility restorer genes *Rf3* and *Rf4* from KMR3R into partial restorer Swarna. All the improved lines of Swarna possessing *Rf3* and *Rf4* genes showed complete fertility restoration with higher grain yield heterosis and can be utilized directly in the hybrid rice breeding.

7. TRANSGENIC DEVELOPMENT AND UTILIZATION IN RICE

Apart from MAS, transgenic approach has also a great role to play. Some of the new genes have been introduced in to rice in order to improve the nutritional quality with higher health benefits. To this end modification of rice grain nutritional composition have been done improving micronutrient content, vitamins and some important essential amino acids via bio-fortification. Folate biofortified rice was developed to meet daily requirements of folate through rice prevents birth defects (De Steur et al., 2014). Another well-known transgenic rice which received the "Patents for Humanity Award" in 2015 was golden rice rich in â-carotene to address the vitamin A deficiency leading to early blindness in children (http://www.goldenrice.org/). The development of golden rice underwent in

two phases, where in phase one, the gene from Daffodils was introduced and in second phase one gene from maize and another from Erwinia were introduced to rice to increase the bio-availability of vitamin A to children and pregnant women through rice (Dash et al., 2016). In addition, transgenic rice is also developed for pharmaceutical purpose, to produce therapeutic proteins, cytokines and vaccines which helped in treating infectious diseases allergy and even treating Alzheimer disease (Azegami et al., 2014). *Fritin* gene from soybean has been transferred in to rice in order to improve rice grain iron content. Rice transgenics have been developed to improve micronutrients content in rice to combat hidden hunger. Significant efforts through transgenic approach have been made to reduce phytic acid content in grain which is a major anti-nutritional factor. Application of transgenic techniques to improve rice grain quality lead to compromise in the yielding ability of rice in many cases including golden rice. Several transgenic rice varieties have been developed; however their large application in to field level is not yet advanced due to GMO monitoring issues in at different stages in different countries.

Several attempts have been made to improve yield through transgenic approach in rice but the successes was very limited (Paul et al., 2018). Modification in expression pattern of *OsPIN5b* gene which encodes ER-localization protein results in higher tiller number, vigorous root growth and increased panicle length lead to increased yield (Lu et al., 2015). Liu et al. (2015) over-expressed *BG1* gene that play important role in increasing grain size and indirectly improved grain production. The GM rice developed by Zhang et al. (2013) by over-expression of miRNA OsmiR397 resulted in more branching in panicle and increased grain size lead to improved yield up to 25% under field trail. Yoon et al. (2020) developed a transgenic rice that overproduces Rubisco - a key enzyme in photosynthesis lead to higher yield production with increased N-use efficiency. Modified genes responsible for increasing photosynthetic activity and N-use efficiency that increased biomass accumulation thereby increase the yielding potential of transgenic rice. Joshi et al. (2020) used a fusion gene from E. coli for developing transgenic rice that can tolerate sodicity in conjunction with drought and salinity tolerance characters along with high yield.

Rice transgenic has also been developed to address the issues of biotic stress like insect resistance and disease resistance. Xu et al. (2018) developed transgenic rice by expressing fusion protein of Cry1Ab and Vip3A toxin which were highly resistant to Asiatic rice borer and rice leaf folder without compromising yield levels. The lower levels of *Cry2AX1* was found effective against rice leaf folder larvae (Manikandan et al., 2016). Ling et al (2016) developed transgenic rice with cry2A* gene via marker free transgenic approach, resulted

transgenic rice plants were found resistant against stripped stem borer, yellow stem borer and leaf folder. Similarly, transgenic rice plant resistant against rice blast disease was developed by inserting *mohrip1* and *mohrip2* genes. These transgenic rice plants showed higher resistance to rice blast disease and stronger tolerance to drought stress.

Significant achievements have been made in transgenic rice development, but utilization of these findings is still limiting due several factors including regulatory issues. Only few countries allowed transgenic crop cultivation. However, as a science and as a technology transgenic approach is found successful with respect to many target crops and traits. In case of rice a major food crop, the biofortification through genetic modification will help enhance the nutritional security. There are several successful examples of transgenic rice targeting different traits; however, there is need to thorough analysis of stability of transformation events and rapid transfer into adapted rice backgrounds through breeding approaches to reap yield benefits.

7. 1 Physiological approach: C4 rice

It is well documented that, the C4 plants do have higher photosynthetic efficiency under both favourable and challenging environmental conditions. In simple terms, C4 plants are excellent for managing or utilizing the overall resource of the plant under normal and stressed conditions. Due to the promiscuous nature of the enzyme Rubisco, its carboxylation and oxygenation capacity is highly dependent on many external factors, which in turn determine the fate of the overall photosynthetic efficiency in plants (Hibberd and Quick, 2002). The oxygenase function of Rubisco results in significant loss C-skeleton fixed via C3 cycle through the process of photorespiration (Sage et al., 2012). In C3 plants, photorespiration exceeds more than 25% under current atmospheric CO_2 concentration and it is expected to rise to 40% when the atmospheric temperature exceeds 35°C as under high-temperature conditions, the solubility of CO_2 decreases more than that of the solubility of O_2 (Sage et al., 2004).This indicates, high temperature favours the oxygenase activity of RuBisCO, which results in a reduction in carbon fixation and increased loss of photo assimilated CO_2 through the process of photorespiration in C3 plants (Andersson, 2008). To overcome this inherent nature of the RuBisCO enzyme, the C4 photosynthetic mechanism had been evolved in C4 plants. Thus, 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. Crops with an enhanced photosynthetic mechanism would better utilize the solar radiation that can be translated into yield. This subsequently will help in producing more grain yield, reduce water loss and increase nitrogen use efficiency especially in the hot environment (Karki et al.,

2013). C4 plants are potentially more productive at higher temperatures typically experienced by rice. To take advantage of this more efficient photosynthetic system at a time when the population and food prices are soaring, there are efforts towards inserting the C4 mechanism such as that found in maize into rice (Rizal et al., 2012). This novel approach to modify the photosynthesis system of rice is a challenging and long term endeavor because the C4 pathway is very complex and many factors controlling the mechanisms are still unknown.

In rice more than 90% of the total chloroplasts are located in mesophyll cells (MCs) within the leaf (Yoshimura et al., 2004); whereas, in C4 plants both MCs and bundle sheath cells (BSCs) possess equal numbers of chloroplasts. To introduce the C4 pathway into rice, more photosynthetic chloroplasts are required in the BSCs than rice has now. This could be done by over expressing the genetic elements that are necessary for the chloroplast development such as Golden2-like (GLK) genes in a cell specific manner by using C4 gene promoters such as phosphoenol pyruvate carboxylase (PEPC) of *Zea mays* for MC specific expression and phosphoenol pyruvate carboxykinase (PCK) promoter of Zoysia japonica for BSC specific expression in rice leaves (Matsuoka et al., 1994; Nomura et al., 2005).

Reduce the vein spacing thereby increasing the vein density in the leaf is another anatomical requirement to develop C4 rice (Karki et al., 2013). Studies on leaf anatomy and morphology have revealed several genes responsible for growth, development or deformities of cells in leaves. A gene *ACAULIS1* was responsible for elongation of leaf cells (Tsukaya et al., 1993). Increase in free vein ending, open venation pattern and rounded leaf structure were caused by *rotunda 1* (*RON1*) (Robles et al. 2010). Besides, to develop C4 rice we need to significantly reduce the activity of Calvin cycle in the MC and greatly enhanced in BSC in rice (Caemmerer, 2010). Not only this, we must reduce the photorespiration process in the MC to control the carbon loss in the futile C2 or glycolate cycle. C4 plants have developed mechanisms to restrict localization and activities of Rubisco in BSCs. MCs spatially prevent the contact between Rubisco in BSCs and O_2 in the intercellular spaces, thus preventing loss of energy through photorespiration. The elimination of photorespiration in C4 rice is a very important step for yield enhancement.

7.2 Other physiological approaches for yield enhancement

Yield potential of any crop is a reflection of its genetic background. And also the morphological and physiological character that comes with that. Yield of rice depends upon the high photo-assimilation which comes from the superior leaf physiology and canopy architectural parameters, higher pre-anthesis carbohydrate accumulation in the sheath, leaf and stem, which helps to maintain

the activity of the spikelets for longer and delayed senescence, which increases biomass production after heading. It also depends on the transport of photo-assimilates to the developing grains and a high sink capacity in terms of large panicle should be there to receive those assimilates (Chang et al., 2016).

The physiological characters which are responsible for higher grain yield are high photosynthetic rate and slow leaf senescence (Katsura et.al, 2007) great biomass accumulation before or after anthesis (Katsura et al., 2007; Yang and Zhang, 2010). A total of 60% to 80% of carbohydrate required for rice grain filling is contributed by the photosynthesis of source leaves during the grain-filling period and the rest are remobilized from culms and leaf sheaths (Li et al., 2017). Yield formation in cereal crop is also closely related to chlorophyll content of the flag leaf during the grain filling stage and abundant chlorophyll in the source leaf is required for biosynthesis of photosynthates (Hisir et al., 2012). Islam et al. (2014) showed a positive correlation of the SPAD values or relative chlorophyll content with grain yield and straw yield of wheat. Qu et al. (2017), identified that leaf photosynthetic parameters such as SPAD and F_v/F_m are highly positively correlated with biomass accumulation, a surrogate of canopy photosynthesis.

Canopy temperature is a good indicator of stomatal conductance and transpiration which relies on stomatal opening with the temperature decreasing as stomata open and as the evaporation rate increases (Furbank et al., 2009). Yan et al. (2012) reported that grain yield and seed setting rate were negatively correlated with canopy temperature at heading stage. Non-structural carbohydrates (NSC) consists of soluble sugars and starch and important for rice grain production. In rice, stem NSC contributes for less than 48% of the grain starch which accumulates before heading. Both grain filling and stem lodging in rice are influenced by culm carbohydrate transport and a moderate NSC transport rate and high SC accumulation in basal culms are the bases of high and stable grain yield in a high yielding rice population (Zhang et al., 2017). They reported greater culm strength in high yielding rice population at Taouan.

8. PRE-BREEDING AND WILD RELATIVES/ANCESTORS

The yield gain realized in the post-green revolution era was the result of high input, intensive cultivation of high yielding rice varieties in the irrigated ecology. The area under irrigated ecology gradually increased over the period and currently accounts for 60% of the total rice area in the country. Being the major ecology for rice cultivation, the average productivity of the irrigated ecology needs to be increased so as to achieve the forecasted rice requirement. But the yield level in the irrigated ecology has been witnessing a plateau in recent years and to break the yield plateau, novel yield enhancing alleles from the entire rice gene pool need to be identified and introgressed in elite genetic backgrounds. Moreover,

novel alleles against the co-evolving biotic stresses and for enhanced nutritional qualities also need to be explored from the wild rice resources.

Several genes/QTL for yield and yield-attributing traits, disease resistance, insect resistance and quality traits have been identified from different wild rice species (Gaikwad et al., 2021). The utilization of identified novel genes/QTL from wild species is limited because of the cross-incompatibility of the cultivated rice with most of the wild rice species. In case of cross-compatible wild species *viz.*, *O rufipogon*, *O. nivara*, the linkage drag and the resultant introgression of non-target deleterious traits limit the practical utilization of novel alleles from these species. The precise introgression of novel alleles from the cross-compatible wild species requires the development of co-dominant, cross-transferable gene-specific markers which produce specific, reproducible bands for easy detection and utilization in marker-assisted backcross breeding programmes.

ICAR-NRRI has developed cross-transferable gene-specific markers for important cloned genes associated with yield-attributing traits and quality traits in rice (Nayak, 2021 and Nayak et al., 2022). These markers are being utilized to study allelic diversity in the wild rice species, for the detection of novel alleles and their introgression in cultivated genetic backgrounds.

9. CONCLUSION

Irrigated rice has contributed immensely for the green revolution and food security. It may continue to serve the humanity as one of the major sources of food and calorie. Continuous efforts are being done to improve the yield levels by breeders and molecular biologists. The yield ceiling in the existing high yielding rice varieties requires special efforts for breaking the yield barrier for meeting food demand of ever increasing population. All the traits contributing grain yield are mostly quantitative in nature where many genes and QTL have intricate interaction with several physiological and bio-chemical processes. In this context, collaborative research efforts are required by breeders, physiologists, molecular biologists and agronomists. In the modern breeding era the systematic use of molecular biology and bio-informatics should support the efforts for successful implementation of smart strategy for further yield enhancement. Yield stability is another factor which decides the success of a variety. This could be possible with incorporation of resistance to different biotic and abiotic stress to make it climate resilient. Similarly, the problems of nutrient use efficiency characters should also be augmented along with bio-fortification to make it complete package. Future thrust should be more focused on few aspects *viz.*, (a) understanding the physiological and molecular basis of morphological traits that are pivotal for higher grain yield; (b) the problems of grain filling in heavy panicle as very high yielding cultures are mostly endowed with such panicles; (c) better understanding of lodging and

vivipary because the modern day HYVs have such tendency; (d) molecular basis of GxE interaction for better yield stability. Moreover, very high yielding cultures need better management to achieve and sustain higher level of productivity, hence, need more attention and research. Therefore, a breakthrough in yield potential and sustainability requires a comprehensive research and improvement of all the aspects without ignoring environment protection and over exploitation of natural resources.

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Challenges in rice improvement for the coastal ecosystems of India under changing climatic scenario

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SUMMARY

Rice production and productivity is severely affected by several biotic and abiotic stresses in coastal ecology. Rice is grown in eastern coastal areas in West Bengal, Odisha, Andhra Pradesh, Tamil Nadu, Puducherry and western coastal areas, Kerala, Karnataka, Goa, Maharastra and Gujarat. Salinity is predominant stress in this ecology. Traditional landraces with tolerance to salinity at varying level such as Korgut, Bhurarata, Karekagga, Pokkali, Bhaluki, Nona Bokra, Kamini, Patnai, etc. are widely cultivated. The *Saltol* QTL was identified using a RIL population generated from the tolerant landrace Pokkali. A QTL, *qSKC1* or *OsHKT1.5* for seedling stage salt tolerance was also cloned. In another accession of Pokkali (AC41585) it was found that *HKT1; 5, HKT1; 1, HAK5 and SOS1* played crucial role in ionic discrimination and selective Na+ movement, resulting in superior reproductive stage salt tolerance. These diverse traditional rice cultivars are used in breeding programme for development of many high yielding varieties including CST1-7, Lunishree, Bhutnath, Jarava, Panvel-3, Luna Suvarna, Luna Sankhi, VTL-8, etc. The rice productivity in coastal areas of India is not only affected by salinity stress at different growth period but also affected by other abiotic stress such as submergence, flash and stagnant flooding by saline water, drought, high temperature and cyclone. The frequency of occurrence of extreme climate due to multiple stresses is increasing under the changing climatic scenario. Under such situation characterization of coastal areas at different Indian states based on occurrence of various abiotic stresses is stated here. Submergence stress is the second most important stress in this ecology. Submergence tolerance both under saline and non-saline floodwater conditions is mostly influenced gas film. But impact of partial submergence (stagnant flooding) with saline water on productivity is much higher than stagnation with fresh water. Land races such as Rashpanjar, Pokkali (AC 39416a), SR 26B, etc. were found tolerant to saline

water under partial submerged condition. Well-developed constitutive aerenchyma in Rashpanjar provided an adaptive advantage during partial submergence with saline water. A variety CR Dhan 412 (NICRA Dhan, Luna Ambiki) tolerant to salinity and moderately tolerant to stagnant flooding has been released for coastal saline areas. A case study was conducted at Sundarbans, West Bengal and it was found that growing stress tolerant rice varieties, farmers could produce 0.44 to 2.14 t ha^{-1} more yield than traditional cultivars even under various abiotic stresses such as salinity, submergence and stagnant flooding.

Keywords: Multiple abiotic stress tolerance, salinity stress, partial submergence stress, climate resilience, coastal saline ecology, rice breeding

1. INTRODUCTION

Rice is cultivated under different ecologies starting from more congenial environment of irrigated conditions to rain-fed ecologies where rainfall pattern determines its productivity. The productivity of rain-fed lowland rice depends on the prevailing environmental factors during the growing season. India has a coastline of 7,516.6 km, of which 5,422.6 km is in the mainland. In coastal rain-fed areas salinity along with other environmental stresses makes the situation worse (Redfern et al., 2012; Dasgupta et al., 2014). Rice cultivation in coastal areas is nature dependent. In this ecosystem, except very rare location in most of the years the rice productivity is always low and far below the average productivity of a nation such as India (Sarangi et al., 2015; Chattopadhyay et al., 2019). Several biotic and abiotic factors influence the rice growth and thus affect the productivity in coastal ecology. The frequent occurrence of coastal disasters as a result of climate change has many impacts in terms of human lives lost, destroyed infrastructure, ecological damage, disrupted social networks, displaced coastal fishing communities from their traditional living and occupational spaces (Senapati and Gupta, 2014). Owing to the greater frequencies of abiotic stresses in the coastal regions, higher productivity in rice is in big danger. These abiotic stresses affect once or at different times or in combination in a growing season of the crop. To stabilize / increase rice yield in coastal areas, tolerance to different abiotic stresses is inevitable (Dasgupta et al., 2014). Among the different abiotic stresses, soil salinity is the most prominent abiotic stress in these areas. Tide gauge readings from four Indian ports from 1993 to 2012 show the rise in sea level by 4.8 cm, increasing about 3.2 mm per year. By 2050 and 2100, sea level is projected to rise by 16 cm and 32 cm, respectively, predicted by the executive director, Dr. V. Selvam of Chennai-based M.S. Swaminathan Research Foundation (https://www.preventionweb.net/news/india-rising-sea-forces-villagers-abandon-rice-farming). Climate-induced sea level rise is a major threat to the country as the 73 coastal districts (out of a total of 593) have a share of 17% of the national population, with nearly 250 million people living within 50 km of the coastline,

reads a 2017 report titled "A report on Problems of Salination of Land in Coastal Areas of India and Suitable Protection Measures" by the Indian government's Central Water Commission.

2. DIVERSITY AND UTILIZATION OF RICE GERMPLASM IN VARIETAL DEVELOPMENT FOR COASTAL SALINE AREAS

A large number of germplasm lines have been collected along the coastal saline areas in India and evaluated by many researchers. Traditionally cultivated local rice landraces and cultivars in coastal area have tolerance to salinity at varying level. Some of the widely used landraces/ cultivars are Korgut, Bhurarata, Kalarata, Karekagga, Pokkali, Chettivirippu, Bhaluki, Rupsal, Nona Bokra, Kamini, Talmugur, Patnai and Getu. These diverse traditional rice varieties are precious genetic resources and used in breeding programme for development of many high yielding varieties including CST1-7, Lunishree, Bhutnath, Jarava, Panvel-3, Luna Suvarna, VTL-8, etc. (Table 1).

Table 1. List of high yielding varieties suitable for coastal saline areas in India

Sl No	Name of Variety	Parentage notifi-	Year of cation	Duration (in days)	Salient Features	Recommended for Cultivation
1	Lunishree	Nonasail Gamma irradiated mutant	1992	145	Tolerant to coastal salinity	Odisha
2	CSRC(S)2-1-7 (IET 13428)	Pankaj/ NC678	2003	-	Semi dwarf; salt tolerant (6.0-8.0dsm^{-1}), resistant to BS, BLB, RTV, ShR, ShB, GLH, WBPH and moderately resistant to SB; Yeild:30-40 Q/ha	Costal saline soils of ZoneIII, IV.V *i.e.*, West Bengal, Odisha, Kerala and Andhra Pradesh
3	CSRC(S)2-1-7 (IET 13428)	Pankaj/ NC678	2003	-	Semi dwarf; salt tolerant (6.0-8.0 dsm-1), resistant to BS, BLB, RTV, ShR, ShB, GLH, WBPH and moderately resistant to SB; Yield:30-40 Q/ha	Costal saline soils of ZoneIII, IV.V *i.e.*, West Bengal, Odisha, Kerala and Andhra Pradesh

Sl No	Name of Variety	Parentage	Year of notifi-cation	Duration (in days)	Salient Features	Recommended for Cultivation
4	CSRC(S)11-5-0-2 (IET 13422) UTPALA	Pankaj/ Jhingasail	2003	140	Semi tall; long slender, resistant to BLB, ShR, ShB and moderately resistant to SB and BPH; Yield:37-48Q/ha	West Bengal
5	Jarava (IET 15420)	B-32-Sela4/ *Oryza rufipogon*/ B29-6	2005	116	Semi dwarf; SB grain, high salt tolerant, suitable for late planting, resistant to blast, neck blast, BLB, RTV, BS, ShR plant hopper and LF; Yield: 33-45 Q/ha	West Bengal, A&N Island, Puducherry
6	VLT-6	Cheruvirittu /IR/Jaya	2005	105-110	Semi tall(115-120cm); grains-MS, tolerant to salinity, acidity and water stagnation; Yield: 35-45 Q/ha	Kerala
7	Bhutnath (CSRC(S) 5-2-2-5) (IET-12855/ 11354	SR26B/ Pankaj	2006	108 Medium maturity	Plant height-112cm LS grain; awn absent, milling recovery 70.25%, resistant to leaf blast, neck blast, ShR, ShB, RTV and BS, moderately resistant to SB, Yield: 40 Q/ha	All India
8	AMAL-MANA (IET 14199/18250)	Pankaj/ SR26B	2008	140	Plant height-140cm extra LS grain, moderately resistant to LB, ShB, resistant to SB, LF,whorl maggot, case worm; Yield: 40 Q/ha	West Bengal, Odisha, Andhra Pradesh
9	VTL-8	IR47310-94-4-3-1X CSR10	2010	115	Semi dwarf (130-135cm); MB grain, pest and diseases are bellow threshold level; Yield:40-42 Q/ha	Kerala

Sl No	Name of Variety	Parentage	Year of notifi-cation	Duration (in days)	Salient Features	Recommended for Cultivation
10	Luna Suvarna (CR Dhan 403)	Mahsuri / Ormundakan	2011	145-150	Semi tall (130cm); MS grain; Resistant to blast, ShB, moderately resistant to BS, YSB, tolerant to LF; Yield:35-40 Q/ha	Odisha
11	Luna Sampad (CR Dhan 402)	Mahsuri / Chakrakanda	2011	140	Short bold grain, tolerant to YSB, BPH, LF and blast and moderately resistant to ShB and BS under natural infestation, 130 cm plant height, tolerant to moderate salinity stress (EC 4-7 dS m^{-1})	Odisha
12	Luna Barial (CR Dhan 406)	Jaya/ Lunishree	2012	150	Short bold grain, moderately resistant to sheath blight and brown spot, plant height-125cm, tolerant to moderate salinity stress (EC 4-7 dS m^{-1})	Odisha
12	Luna Sankhi (CR Dhan 405)	IR31142-14-1-1-3-2 /IR71350	2012	120	Tolerant to salinity	Odisha
13	CR Dhan 412	Savitri/ SR 26B	2021	120	Tolerant to salinity, moderately tolerant to stagnant flooding and lodging	Odisha

Note: BB – Bacterial Blight, BLB - Bacterial leaf Blight, BLS – Bacterial Leaf Streak, LB – Leaf Blight, RTV – Rice Tungro Virus, ShR – Sheath Rot, ShB – Sheath Blight, BPH – Brown Plant Hopper, BS – Brown Spot, GH – Grass Hopper, GM – Gall Midge, GLH –Leaf Hopper, GPH - Green Plant Hopper, GSV – Grassy Stunt Virus, LF- Leaf folder, RH – Rice Hispa, YSB – Stem Borer, SB- Stem borer, WM – Whorl Maggot, WBPH – White Backed Plant Hopper, LB – Long Bold, LS –Long slender, MS – Medium Slender, MB – Medium Bold.

3. VARIATION IN ABIOTIC STRESS PROFILE IN THE COASTAL AREAS OF DIFFERENT STATES

Rice is grown in coastal areas in West Bengal, Odisha, Andhra Pradesh, Tamil Nadu, Puducherry, Kerala, Karnataka, Goa, Maharastra and Gujarat. Apart from salinity, rice frequently faces the other abiotic stresses. Extent of damages sometimes goes high due to multiple abiotic stresses which occur in isolation or in combination in different year and in different location. The frequency of occurrence of multiple stresses is increasing at a higher rate under the changing climatic scenario.

It is being observed from AICRIP testing those new rice genotypes for coastal saline areas lack consistency in performance. Genotypes suitable in one location under severe or moderate salinity stress are not found suitable under such location in next year due to severity of abiotic stress other than salinity. The rice productivity in coastal areas of India is not only affected by salinity stress at different growth period but also affected by other abiotic stress such as submergence, flash and stagnant flooding by saline water, drought, high temperature and cyclone. The frequency of different stresses is progressively going higher due to climate change and therefore, characterization of different coastal location in India must be dynamic in nature.

Coastal West Bengal and Odisha are frequently affected by salinity (1-8 d Sm^{-1}), partial and complete submergence for varying period of time at different crop growth stages due to intrusion of sea water, osmotic stress (due to intermittent dry spell), and cyclonic storm at flowering stage. In general, the salinity stress is relatively low during rainy season due to excess water in the field. But water logging with saline water even with low EC (< 4 d Sm^{-1}) significantly reduce grain yield. Salinity (4-12 d Sm^{-1}) is the prominent stress in dry season and significantly reduces productivity.

In Karnataka, coastal ecosystem is characterized by sea water intrusion occurs to the low-lying water-logged areas, flood prone and ill drained lands. The salinity of the soil varies from season to season with EC of 1.5 to 4.5 d Sm^{-1} during rainy season and 8.6 d Sm^{-1} during dry season. In general, the yield loss in coastal Karnataka is either due to excess rainfall at the beginning of the *kharif* season or due to intrusion of excess amount of brackish water (flood) at any stage of crop growth stages. At the end of the cropping season there may be a dry spell and deposition of salt crystals which causes salinity stress at reproductive stage of the crop resulting in significant grain yield reduction.

Salinity, acidity and submergence are the main abiotic stresses of the coastal areas in Kerala. The soil is acid Sulphate with stiff impervious clay, rich in organic matter. Direct inundation of sea water from the sea during high tide makes the

soil saline. The range for soil pH and EC is 2.62 to 5.97 and 0.1 to 9.8 dS/m, respectively during crop season. In 'Pokkali' areas crop is frequently experienced excess water stress along with salinity stress.

In Goa, rice is exposed to partial to complete submergence along with salinity stress during the early vegetative stage. Later during flowering stage, there is a gradual increase in salinity which goes more than 7dS/M in the month of October. Maharashtra also faces similar kind of situation during rice growing period.

Karaikal district of Puducherry, where PAJANCOA & RI is situated, is encircled geographically by Tiruvarur, Nagapattinam and Mayiladuthurai districts of TN. The cropping season and cropping pattern of Karaikal district remains the same as that of adjoining districts. In order to alleviate the Cauvery water problem, a greater number of bore wells was installed to raise *kuruvai* (June-July) rice. Due to uncertainty in monsoon rains or less quantum of canal water received, recharging of the ground water is drastically affected. This resulted in salt-water intrusion into the alluvial aquifer and hence deep bore wells pump only poor-quality water (1.15 and 4.50 dS/m for E.C; 7.50 and 8.90 for pH; 8.60 and 25.10 for SAR and 1.0 and 13.37 meq/l for RSC). Quality of most of the bore wells falls under highly saline sodic. The same scenario prevails in the adjoining districts of Tamil Nadu in the Cauvery Delta Zone. Short-term submergence regularly affects the rice growing areas in tail end areas of Cauvery delta zone of Tamil Nadu and Puducherry during North East monsoon (*rabi:* October-December). Therefore, rice varieties planted during this period suffer heavy yield loss. Plant type of 110-115 days duration, MS white rice with medium tall and salt tolerant (both vegetative and reproductive stage) are needed for the *kharif* (May-Sept) crop. However, for *rabi* season (Oct-Jan), the breeding targets of submergence tolerance 135-140 days maturity duration, MS white rice with medium tall and anaerobic germination, stagnant flooding are immportant.

4.ADVANCEMENT ON THE GENETIC BASIS OF SALINITY TOLERANCE AND ITS IMPROVEMENT

Genetically, salinity tolerance is a complex quantitative trait (Foolad and Jones, 1993) which makes it difficult for plant breeders to select for improved genotypes due to low expressivity, heritability and large effects of environment on the trait. Still, genetic resources are vital for any trait and intraspecific selection has contributed to improved tolerance in rice (Akbar and Yabuno, 1977). Over the past decade, research efforts have focused on the bi-parental mapping and identification of QTL contributing to salt stress tolerance and transferring to high yielding background through marker assisted selection (Singh et al., 2007; Haq et al., 2010).

The *Saltol* QTL in rice was identified by employing a RIL population generated from the tolerant landrace Pokkali and the highly sensitive IR 29 by AFLP genotyping. Further, it was shown that the *Saltol* QTL contributed to 43 % of variation for seedling shoot Na^+/K^+ ratio (Bonilla et al., 2002). A total of 11 QTL including major QTL for shoot K^+ concentration on chromosome 1 (*qSKC-1*) and shoot Na^+ concentration on chromosome 7 (*qSNC-7*) were identified from an F_2 population derived from a cross between tolerant *indica* rice Nona bokra and sensitive *japonica* genotype Koshihikari (Lin et al., 2004). These QTL were found to influence the root and shoot Na^+ and K^+ accumulation as well as survival under salt stress. In rice alone, more than 100 QTL for salt stress tolerance have been mapped across the 12 chromosomes, mainly at seedling stage (Kumar et al., 2015). However, cloning of QTL is still a rate-limiting step, mainly due to difficulties in fine mapping and defining precise QTL limits. A single QTL, *qSKC1* or *OsHKT1.5* for seedling stage salt tolerance was fine mapped and successfully cloned (Ren et al., 2005). Haplotypes of HKT1.5 and HKT2.3 associated with high salinity tolerance revealed through haplotype analysis of 299 wild rice accessions (Mishra et al., 2016). A high-density GBS-based SNP linkage map, validated 14 reported QTL for shoot potassium concentration and other traits and identified several epistemic QTL which significantly contributed to the variation of the traits. Genes located within QTL intervals indicated that ion transporters, osmotic regulators, and protein kinases might contribute significantly in seedling stage salt tolerance (De Leon et al., 2016). A total 28 QTL for salinity tolerance at seedling stage in relation to Chlorophyll fluorescence and photosynthetic efficiency were detected using a biparental mapping population. Thirteen QTL for Fv/Fm (maximum quantum yield in PSII), 5 QTL for *ØEo* (efficiency with which a PSII trapped electron), *8* QTL for *ÖEo* (quantum yield of electron transport flux from Q_A^- to PQ pool), one each for *ÖRo (*quantum yield of reduction in the end electron acceptors of PSI) and PI_{ABS} (performance index on absorption basis) along with 2 QTL for *SSI-Na-K* (stress susceptibility index for Na/K concentration ratio in shoot) were detected (Chattopadhyay et al., 2020a).

A study showed regulated and selective movement of Na+ to flag leaf and developing panicle resulted in reduced spikelet sterility and panicle degeneration in Pokkali (AC41585). It was found *HKT1; 5, HKT1; 1, HAK5 and SOS1* played crucial role in ionic discrimination and selective Na+ movement in Pokkali, resulting in superior reproductive stage salt tolerance (Chakraborty et al., 2019). For salt tolerance at reproductive stage, 16 QTL for pollen fertility, Na^+ concentration, Na-K ratio in the flag leaf on chromosome 1, 7, 8, 10 (Hossain et al., 2015) and 35 QTL for different yield attributing traits (Mohammadi et al., 2013) were identified. But none of them could be validated, fined mapped and cloned for deployment in salt tolerance breeding programme (Chattopadhyay et

al., 2013). Due to population specificity and limited scope of using diverse tolerant germplasm lines in bi-parental mapping, genome wide association mapping exploiting large scale single nucleotide polymorphism have been practiced to capture natural variations in loci and allelic variations for candidate genes of complex abiotic stresses such as salinity. Using custom-designed array based on 6000 SNPs, Kumar et al. (2015) identified 20 loci associated with Na-K homeostasis. They found *Saltol* as the major salt tolerance QTL not only for seedling stage, but also for reproductive stage in relation to Na^+-K^+ ratio in leaves. New QTL were also found on chromosome 4, 6 and 7. Nine QTL were found consistent over the years. *qDEG-S-2-2* and *qDEG-S-4-3* for spikelet degeneration, *qSSI-STE-2-1, qSSI-STE-2-2, qSSI-STE-3-1, qSSI-STE-4-1, qSSI-STE-11-1 f*or stress susceptibility index for spikelet sterility, *qSSI-Grain-2-1* for stress susceptibility index for number of grains/panicle and *qK-S-1-1* for K^+ concentration in flag leaf. SSR markers RM17016 and HvSSR06-63 on chromosome 1 and 6, respectively, detected to be associated consistently with spikelet sterility under salinity stress at reproductive stage (Chattopadhyay et al., 2020b). There is a need to direct research efforts towards identification of genes governing tolerance to salt stress which in turn would aid in development of perfect gene-based markers and pyramiding of multiple QTL in a single genetic background so as to provide tolerance under diverse stress environments.

5. COMBINED STRESS- SALINITY AND STAGNANT FLOODING / SUBMERGENCE: BASIS OF TOLERANCE AND ITS IMPROVEMENT

Flooding with salty water is posing greater threat due to climate change and other associated happenings to rice grown in coastal plains (Wassmann et al., 2004; Schumahcer 2006; IPCC 2007; Garcý´a et al., 2007). Flooding with salinity is a common problem (Flowers and Colmer 2008). Intrusion of sea water may displace millions of people from coastal plains and is the direct threat of the food security of the poor and marginal people reside along the coastal belts (Wassmann et al., 2009). In coastal areas two types of flooding are seen, 1) submergence- where both roots and shoots are under water for a short period of about 2 weeks, and 2) partial submergence or stagnant flooding- where certain portions of the shoots remain above the flood water surface and rests are under water. Probability is high that flooding may occur with salty water. Normal rainfall decreases salinity problem whereas deficit rainfall increases the problem. Salinity reduces plant growth and plants become weak. Salinity followed by flooding is more vulnerable. The already weak rice plants die out early compared to the healthy plants (Sarkar and Ray, 2016). Salt tolerant plants maintain their health better under salt-stress compared to intolerant plants, so improving salt-tolerance is imperative for rice under coastal plains even to counteract the deleterious effects of flooding (Sarkar et al., 2019). Our traditional landraces in coastal area may not be high yielder but

are treasurer of tolerance to salinity and other different stresses prevailed since long time during rice cultivation. Some of them are tolerant to multiple abiotic stresses.

6. SUBMERGENCE AND SALINITY

Submergence tolerance is mainly controlled by submergence tolerance gene, *Sub1A1* (Fukao and Bailey-Serres, 2008), however, flood water quality like light, turbidity, concentrations of oxygen and carbon dioxide and even the concentration of ethylene in flood water influence the survival under submergence (Setter et al., 1997; Panda et al., 2006; Das et al., 2009). Damage of plants under salinity depends on the concentrations of Na^+, Cl^-, K^+ and Na^+: K^+ ratio. Transpiration ceases under submergence and therefore restricts the entry of ions whereas direct contact of shoots with flood water containing higher concentrations of salt broadens the entry of ions Floodwater under natural condition especially during day time is hardly anoxic rather sometimes super saturated with oxygen whereas during night floodwater is hypoxic in nature (Setter et al., 1995; Ramakrishnnya et al., 1999; Colmer et al., 2014). During darkness pO_2 in root declines sharply (0.24 kPa), yet it never reaches in anoxic state. Under light, however root pO_2 is high (14 kPa). Under natural flooding floodwater is not at all anoxic, though oxygen concentrations vary greatly during 24 h time period starting from very low to very high. It might influence the entry of ions and therefore survival of plants under saline floods. Under submergence gas film is created between the surface of the leaf and surrounding floodwater (Winkel et al., 2014; Chakraborty et al., 2020). This restricts the entry of salts and helps in continuation of gas exchange between plant and surrounding floodwater (Tamang and Fukao, 2015). Gas films impart submergence tolerance both under saline and non-saline floodwater conditions. Sarkar and Ray (2016) observed no difference between saline (12 dS m^{-1}) and non-saline (0.17 dS m^{-1}) floods; FR13A, tolerant to submergence but susceptible to salinity showed similar survival under both the situations. Submergence is a short-term consequence with 1-2 weeks duration. Entries of injurious concentrations of Na^+ and collapses of gas films take time and therefore submergence tolerant cultivar FR13A survived the situations but cultivars susceptible to submergence did not.

7. WATER LOGGING AND SALINITY

Lowland rice is tolerant to stagnant flooding (Kuanar et al., 2017) yet it suffers greatly under saline water stagnation (Prusty et al., 2018). Partial submergence (depth of water 45 cm) with salinity (12.0 + 0.2 dS m^{-1}) was imposed on forty-five days old seedlings and within 10 days symptoms of salt injury appeared though it is stated that rice is comparatively tolerant to salinity at vegetative and tillering stages (Munns and Tester, 2008;). Like salinity stress under combined effect of

salinity and stagnant flooding tolerant plants try to inhibit the damage of pigment concentrations, maintain better chloroplast structural and functional ability (Zheng et al., 2009; Ray et al., 2017; Prusty et al., 2018), improve oxygen transport through formation of aerenchyma tissues and antioxidant systems (Flowers and Colmer, 2008; Zheng et al., 2009; Haddadi et al., 2016). Genotypes tolerant to combined stresses though are now well characterized (Prusty et al., 2018). Phenotyping technique has been improved (Pradhan et al., 2019). Yet, development of high-yielding rice varieties tolerant to combined effect of salinity and water stagnation is still at stake. Among lowland rice cultivars which were tolerant to salinity at seedling stage showed tolerance to saline-stagnant flooding stress, though the degree of tolerance varied among them. A land race Rashpanjar was found tolerant to saline water under partial submerged condition. Well-developed constitutive aerenchyma in Rashpanjar provided an adaptive advantage during partial submergence due to saline water flooding in rice as the key process of induced aerenchyma formation is hampered in the presence of salinity stress coupled with partial submergence (Chakraborty et al., 2021).

8. CLIMATE SMART VARIETIES AND LIVELIHOOD IMPROVEMENT

Due to rising sea-level, intrusion of sea-water further in inland, increase frequencies of cyclonic storm. It is not uncommon that rice comes across salt damage along with flooding with saline water during the growing period (Wassmann et al., 2009; Redfern et al., 2012; Dasgupta et al., 2016; Pradhan et al., 2019). The country with greater coastal area would suffer more due to non-availability of high yielding salinity-waterlogging tolerant rice cultivars (Dasgupta et al., 2016). Livelihood of these peoples mainly depends on the cultivation of rice. Their food security is a great concern (Dasgupta et al., 2016). Situation is grim but till date,

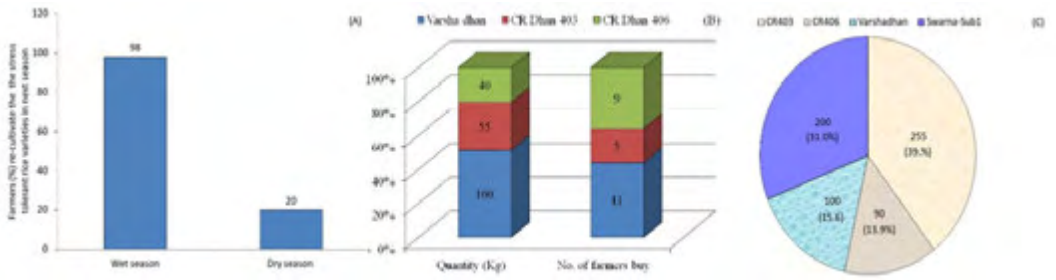

Fig. 1. Impact of cultivation of stress tolerant rice varieties in Sundarbans area. A) Percentage of farmers convinced to re-cultivate the stress tolerant rice during wet and dry season, B) Numbers of farmers purchased the seeds from other farmers by seeing the performances of stress tolerant rice, C) Quantities of seeds purchased by farmers from farmers group / NGOs.

the condition is not improved much. A case study was conducted at Sundarbans, West Bengal a highly populace place where rice is mainly grown during the rainy season. Most of the farmers are poor. Improving rice productivity is directly helpful to these people to secure food, employment and overall social security. It was found that growing stress tolerant rice varieties, farmers could produce 0.44 to 2.14 t ha^{-1} more yield than traditional cultivars even under various abiotic stresses such as salinity, submergence and stagnant flooding (Chattopadhyay et al., 2019). Additional produce of rice would reduce the dependence of poor and marginal farmers on forest. Little financial goodness to poor people has great consequence on society in relation to education and health. Seeds produced locally were most acceptable to small and marginal farmers and were more accessible to them (Fig. 1). Seed production at village level would be more useful to small and marginal farmers. Therefore, stress tolerant rice varieties have been proved an armoury to fight against insecurity to food and livelihood.

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Climate-Smart Rice Breeding: Progress and Challenges for the Rain-fed ecologies in India

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SUMMARY

The effects of climate change are more stressful for higher rice production in recent years and likely to be more intense in the coming years too. Rice cultivation under rain-fed ecology faces several challenges for higher production. Suitable rice breeding techniques in varietal improvement programmes will play a pivotal role for enhancing food grain production under climate change to feed the growing population. Simultaneous incorporation of multiple stress resistance/tolerance genes/QTL will be crucial for developing climate-smart rice varieties. Searching and deploying the relevant genes and QTL for the resilience traits in the breeding programs would be more crucial to breed elite climate-smart rice varieties. It is apprehended that these climate-smart varieties will be adapted under the adverse climatic events and other environmental stresses. Recent advancements in search of genes and QTL for controlling the climate resilience has helped to develop and release climate-smart varieties for the rain-fed upland, shallow lowland, semi-deep and deep-water rice ecologies. These aspects have been discussed at length for attaining higher productivity in such ecologies. Improvement for abiotic stresses tolerance mainly for submergence, water logging, anaerobic germination, drought, salinity, lodging resistance, cold, seed dormancy and biotic stresses namely tolerance to brown plant hopper, gall midge, leaf folder, bacterial leaf blight, blast, sheath blight, false smut, and others are also discussed.

Keywords: Abiotic stress, biotic stress, climate resilience, climate smart, rain-fed rice, upland rice, shallow lowland rice, water logged rice

1. INTRODUCTION

Rice is the most widely consumed food for about half of the global population. India has the largest rice area but the growing environments are extremely diverse and challenging. Rice is the most important food crop worldwide and grown as a staple food. It plays a vital role in the national food and nutritional security. Similarly, it is a staple food for three fourths of Indian population and continues to hold the key to sustain food sufficiency in the country. Being the staple for 65%

of the population, at the current level of per capita availability rice production is required to go up by 70% - from 105 to 190 million tons of milled rice by 2050. We would be, therefore, requiring adding annually not less than 3.0-3.5 million tons of milled rice to sustain the present level of self-sufficiency in rice. It is therefore, a challenging task to achieve this targeted production levels in the next few decades as increase in productivity has to come from the declining and degrading resource base in terms of land, water and other inputs and demand for environmentally sound rice production practices (Siddiq., 2013). Rain-fed rice is cultivated in an area of 22 m ha constituting about 45% of total rice areas in the country. The productivity of rain-fed rice is very low due to extreme diversity in growing conditions. Rain-fed rice ecology is broadly divided into drought prone upland, favorable lowland and unfavorable flood prone lowland and salinity affected areas. These rice growing areas are mostly located in the eastern parts of the country and face various biotic and abiotic stresses. The major abiotic stresses of the rain-fed ecologies are submergence, drought, low and high temperature, high wind, vivipary germination and soil toxicity related problems. Among the biotic stresses, bacterial blight, sheath blight, false smut, stem borer, brown plant hoppers are main biotic constraints for higher production. The frequency and intensity of these stresses are increasing due to the effect of unpredictable climate change. Among various abiotic stresses, submergence, drought, salinity and high wind speed cause heavy loss to the rice farmers in these regions. Minor diseases and insect pests are also becoming very prominent and causing heavy loss. Therefore, climate-smart varieties are highly required in these regions to tackle the challenges faced by the producers for sustainable rice farming.

The adverse effects due to climate change are the most serious challenges now mankind is facing. The stresses caused by the climate change are great limitation to higher production from rain-fed rice ecosystems and threatening sustainability and livelihood security. The global warming due to the anthropogenic activities generating more carbon dioxide and other greenhouse gases in the atmosphere are threat to mankind. In addition, the extreme events are highly related to water availability in the country. The current per capita availability of water is expected go down to 1,000m^3/capita/year by 2050 from the current level of 1,600 m^3/capita/year. Rice cultivation consumes majority of water in the country and future climate with water non-availability may greatly limit the rice cultivation in the country. Thus, rice production for resource poor farmers of the rain-fed regions should have resilient production technologies to tackle the adverse effects in future.

The quality and quantity of food grain production is continuously affected by the climate change. Multiple stresses are causing problems in achieving high yield from the rain-fed farming. Thus, the combined effects of stresses on rice

plants at different growth stages are becoming a challenge for higher production. The occurrences of flash flood and drought during the different growth stages of rice crop are now common in eastern India which reduces rice production drastically. Further, the frequency and intensity of such conditions are likely to be more in future. The prevalence of various biotic stresses is also expected to damage more under the climate change. The global temperature has increased by 0.6^0C over the last 100 years. The rise in temperature is expected to be more and the rise will be 0.5-2.8^0C by the end of this century. With rise in 1^oC temperature, a reduction of 10% crop yield may occur (Peng et al., 2004). Thus, combining traits like drought, flooding or high temperature tolerance with biotic stress resistance are very important for developing climate-smart rice varieties for sustainable rice production in the country.

India has vast costal rice area surrounded by sea from three directions. Salinity is a problem in rice cultivation in such areas. Besides salinity, water logging, high wind speed, vivipary germination at maturity and even intermittent drought spell are observed during various growth stages of wet season rice in these areas. The intrusion of salt water is rising and encroaching more areas under salinity. The east coastal regions comprising Odisha and West Bengal have about 60 % of the coastal ecology. Cyclonic storm with high wind speed are common occurrences in these regions and many times lodging of matured plant causes huge loss to the cultivators. Continuous rainfall during crop maturity stage forces seed germination in the standing crop of non-dormant varieties and makes severe loss to the farmers. Incorporation of these resilience traits into the high yielding varieties will be more suitable and profitable to the coastal rice farmers. The rain-fed lowland ecologies cover around 14 mha of which 92% are located in the eastern region of the country. Depending upon the water depth and duration of water logging in the lowland ecology during growth stages of rice, it has been classified into rain-fed shallow lowland, semi-deep, deep and very deep water or floating type. The rain-fed shallow lowland is characterized by water accumulation of 0-50 cm that faces frequent flash flood and intermittent drought. As these areas are not suitable for growing most of the other economically important crops, therefore, development of high yielding climate resilient varieties of rice under such harsh ecologies becomes very important. In future, rice production needs to be increased from lesser land area. In fact, with the increasing scarcity of water for rice cultivation, the future thrust for growing rice must get focus on such ecology. This chapter deals with advancement, prospects and constraints of rice improvement under the scenario of climate change for achieving higher productivity. Improvement for abiotic stresses tolerance namely submergence, drought, water logging, anaerobic germination, salinity, lodging resistance, cold, seed dormancy and biotic stresses like tolerance to brown plant hopper, gall midge, leaf folder, bacterial leaf blight, sheath blight, false smut, and others are also discussed.

2. CLIMATE-RESILIENT RICE BREEDING FOR THE RAIN-FED ECOLOGIES

2.1 Climate resilient breeding for the upland ecology

Upland rice ecology covers about 6.0 mha area in India. Major growing areas are in eastern and north-eastern states of the country. The ecology is mainly monsoon dependent for rice production. The productivity of upland rice is low mainly due to soil moisture stress, weeds, poor soil nutrient status and diseases incidences (blast and brown spot). More research efforts are required to understand fully about the complexity of drought tolerance trait and due to such situation the progress in breeding for tolerance is also slow. Farmers either grow early duration high yielding sensitive varieties or low yielding tolerant landraces in the drought prone areas. Thus, breeding efforts are focused on development of high yielding and drought-resilient rice varieties with built-in resistance to major insect pests and diseases.

Breeding for drought tolerance through selection of yield component and secondary traits was less effective. However, direct selection for grain yield under the stress is more heritable under normal condition and resulted in significant gain in yield under the stress (Bernier et al., 2007; Kumar et al., 2008). Pure line selection was initially performed from the elite tolerant landraces to develop drought tolerant high yielding varieties in India. Prominent selections were N22 from Rajbhog, BR19 from Brown Gora and PTB10 from Thavala Kannan. Good donor parents were identified and used in the breeding program. Short duration and drought tolerant varieties such as Bala, Annada, Annapurna, Cauvery, Parijat, Pusa2-21, Rasi and Kanchan were released. However, semi-tall varieties are preferred for weed competitiveness rather than dwarf varieties and semi-tall varieties namely Kalinga III, Vandana, Anjali, Virendra, CR Dhan 40 and Sahbhagidhan, lndira Barani dhan-1 and Shushk Samrat were released. High priority was given for high yield under normal condition and also under drought conditions. Sahbhagi dhan, Satyabhama (CR Dhan 100), Ankit (CR Dhan 101) and Santa Bhima (CR Dhan 102) were resilient under drought and showed higher productivity under normal condition.

Previous studies on molecular genetics identified numerous QTL (quantitative trait loci) linked to different physiological and biochemical traits (Alexandrov et al., 2014; Todaka et al., 2015; Dixit et al., 2017; Barik et al., 2019), but failed to identify any gene that regulates these traits, because of low mapping resolution and weak phenotypic effect (Singh et al., 2016; Bin Rahman and Zhang, 2016). Identifying this QTL related to selective trait helps in stress screening programs for plants (Vikram et al., 2011). Many QTL linked to different physiological and growth traits under drought have been identified and used

extensively for selection of tolerant rice genotypes (Venuprasad et al., 2012; Vikram et al., 2011, 2016; Dixit et al., 2017; Vinod et al., 2019). A prominent QTL, *qDTY1.1* was identified for improvement of yield under drought stress (Vikram et al., 2011). The other useful yield QTL identified from different mapping populations were *qDTY2.1* (Dixit et al., 2012), *qDTY2.2* (Dixit et al., 2014), *qDTHI2.3* (Lin et al., 2007), *qDTY3.1*, *qDTY6.1* (Venuprasad et al., 2012), *qDTR8* (Ramchander et al., 2016), *qDLR8.1* (Qu et al., 2008), *qDTY9.1A* (Dixit et al., 2012) and *qDTY12.1* (Mishra et al., 2013). Hence using these linked QTL will be useful resources for molecular screening of drought tolerance in rice. Genetic studies for several traits such as root characters, osmotic adjustment, cell membrane stability, relative water content, leaf rolling and stomatal conductance were reported for drought tolerance in rice (Venuprasad et al., 2012; Dixit et al., 2017). Genetic mapping of morpho-physiological traits during reproductive stage drought stress detected QTL such as *qRWC, qLR9.1, qLD9.1, qHI9.1, qSF9.1, qRWC9.1, qRCC1.1, qCHLa1.1* and *qPRO3.1* for leaf rolling, leaf drying, harvest index, spikelet fertility and relative water content, respectively (Barik et al., 2018; Barik et al., 2019; Barik et al., 2020). The first drought tolerant variety through marker assisted selection was IR64 Drt1 by pyramiding *qDTY2.2* and *qDTY4.1* in the background of IR64 and released in the year 2014. In another breeding programme, yield QTL such as *qDTY9.1, qDTY2.2, qDTY10.1* and *qDTY4.1* have been incorporated into IR64 variety by adopting marker-assisted backcross breeding approach (Singh et al., 2016). A Malaysian rice variety MR219 was converted to drought tolerant by pyramiding of QTL, *qDTY2.2*, *qDTY3.1*, and *qDTY12.1* (Shamsudin et al., 2016). Drought yield QTL such as *qDTY3.1*, *qDTY6.1*, and *qDTY6.2* were incorporated into TDK1 rice variety for getting high yield under drought (Dixit et al., 2017).

Rice root is usually a shallow rooting type compared to other cereal crops and hence is sensitive to moisture stress. Deep rooting may help plants to avoid drought-induced stress by extracting water from deep soil layers (Yoshida and Hasegawa, 1982; Fukai and Cooper, 1995). Vertical root distribution in rice showed low moisture stress to plants compared to shallow rooting type (Kondo et al., 2000). The genetic control of deep rooting in rice was later confirmed (Uga et al., 2011). To improve upland rice for drought avoidance, introducing the deep-rooting characteristic into shallow-rooting cultivars is considered one of the most promising breeding strategies. So far, only one quantitative trait locus (QTL) responsible for deep rooting namely *Dro1* on chromosome 9 has been cloned in rice (Uga et al., 2011, 2013a). *qSOR1*, a QTL for soil surface rooting on chromosome 7 (Uga et al., 2012), *Dro2* on chromosome 4 (Uga et al., 2013b) and *Dro3* on chromosome 7 (Kitomi et al., 2015) have been mapped. Genotypic variation contributes considerably for root growth angle in rice cultivars with functional *Dro1* allele along with other major QTL such as *Dro2, Dro4* and *Dro5*, and by several

additional minor QTL (Kitomi et al., 2015). Hence, screening of germplasm lines with deep rooting and subsequent utilization as donor in the rice improvement program in the superior background is essential for upland rice. The yield limitation in upland rice is also due to low phosphorus availability.The phosphatic fertilizer's cost is high in India and farmers are usually not applying the fertilizer under upland condition. Rice genotypes having better uptake efficiency under P-deficient soils can be very useful and adaptation of such varieties require no extra cost for cultivation (Aziz et al., 2006). Hence, the high yielding popular varieties need to be improved for P-uptake for better yield under low P condition. P uptake is controlled by a major QTL, Phosphorous uptake 1 (*Pup1*) which shows 78.8% phenotypic variance and located on the chromosome 12 (Wissuwa and Yano, 1998; Wissuwa et al., 2002). The upland landraces such as Surjamukhi, Karni, Lalsankari, Tepiboro, N22 and Bowdel are good donor lines for *Pup1* QTL (Pandit et al., 2018). Dular, Tepiboro, Surjamukhi, Bamawpyan, N22, Dinorado, Karni, Kusuma, Bowdel, Lalsankari and Laxmikajal contain *Dro1* and *Dro2* QTL (Pandit et al., 2020). Recently, *Dro1* and *Pup1* QTL have been pyramided in the background of popular varieties Lalat, Naveen, Satbadi and MTU1010.

2.2 Climate-resilient rice breeding for the rain-fed shallow lowland ecology

Farmers face several biotic and abiotic challenges which limit rice production of the shallow lowland ecology. The common problems of flash flood and intermittent stagnant flooding are associated with the shallow lowlands of eastern India. The farmers suffer heavy loss by growing high yielding but sensitive varieties which results in complete crop failure under this ecology. Under submergence condition, gas diffusion rate through leaf tissue reduces the oxygen uptake and forces inefficient carbohydrate metabolism for which plant faces multiple internal stresses, essential for plant growth and survival. Further, submergence by turbid water reduces light availability that inhibits photosynthesis and leaf gas exchange. However, rice scientists have identified useful QTL which confer tolerance to the submergence stress. The screening for submergence tolerance led to the discovery of two varieties, namely FR13A and FR43B. These varieties were collected from Odisha, located in eastern India. The designation of these donor lines as FR is for "flood-resistant" (Vergera et al., 1976). These cultivars were developed through pure line selection from the traditional local varieties. FR13A was selected from Dhalputia, while FR43B was selected from Bhetnasia. Further screening at different environments and seasons later confirmed the strong tolerance of FR13A (HilleRisLambers and Vergara, 1982). Based on the available literature, FR13A is the most recognized submergence tolerant landrace and has been widely used as a donor parent for submergence tolerance breeding programs. Similarly, it has been widely used for quantitative trait locus (QTL) mapping and subsequent map-based cloning of the *Sub-1* gene. IR40931-26 used for the improved source of *Sub-*

1 was derived from FR13A, and fifty days old plants of this line showed prolonged submergence tolerance under green house and field conditions. A major QTL, *Sub-1A* is located on the chromosome 9 and very effective for submergence tolerance response for about 2 weeks (Xu et al., 2006). The QTL contributes ~70% of the phenotypic variance for submergence tolerance. This major QTL has been transferred to many popular varieties *viz.*, Swarna (Neeraja et al., 2007), KDML105 (Luo & Yin, 2013), BR11 (Iftekharuddaula et al., 2015), PSB Rc18 (Septiningsih et al., 2015), Ciherang (Septiningsih et al., 2015; Toledo et al., 2015), IR64 (Mackill et al., 2012; Collard et al., 2013) and CR1009 (Robin et al., 2019). The popular varieties such as ADT 46, Bahadur, Ranjit, HUR 105, Sarjoo 52, Pooja, Pratikshya, MTU 1075 and Rajendra Mahsuri were also converted into submergence tolerant using marker-assisted backcross breeding (Bhandari et al., 2019). *Sub1* QTL has also been transferred into Gayatri, Sarala, Ranidhan and Varshadhan which are now under advance stage of testing. Using a high level of precision in MABC, the essential features of the mega-varieties (i.e. yield, grain quality, agronomic traits) were retained (Iftekharuddaula et al., 2011; Neeraja et al., 2007; Septiningsih et al., 2009; Singh et al., 2009). In recent years, the *Sub1* varieties have been thoroughly field tested, and indicated no negative effect of *Sub1* on other traits including yield under favorable conditions. Furthermore, these *Sub1* introgressed mega-varieties have been widely adopted by farmers (Mackill et al., 2012; Collard et al., 2013; Dar et al., 2018; Pradhan et al., 2018).

Accumulation of more poly-amines in the tolerant genotypes under drought stress show more photosynthesis, low water loss and better osmotic adjustments (Capell et al., 2004). More proline content is also associated with the tolerant genotypes and helps in maintaining leaf water potential and turgor pressure (Kandowangko et al., 2009). Therefore, proline accumulation in varieties may be a selection for drought tolerance breeding in rice (Dien et al., 2019; Barik et al., 2020). ABA increases during terminal drought stress results closure of stomata and reduces transpiration (Ji et al., 2011). ABA production during drought stress show a positive correlation with pre-stored carbon's remobilization in stem, leaves and roots (Yang and Zhang, 2010). Deployment of ABA-dependent genes namely *OsNAP* and *OsNAC5* increased yield under reproductive stage drought stress (Liang et al., 2014). Under drought, ethylene's concentration has been shown to increase in rice grain at early grain-filling stage and decrease during grain development (Yang et al., 2004). The ethylene concentration is negatively correlated with the grain-filling rate (Zhu et al., 2011). Proline, sugar and starch content increases under the drought stress (Dien et al., 2019). Membrane stability, increase Leaf rolling score, relative water contents in leaf, deeper and thicker roots are important parameters for selection of drought tolerant progenies.

Inheritance of grain yield is very complex and polygenic in nature. Harvest of high yield is dependent on suitable environmental condition during the crop growth stages. Drought stress particularly at reproductive stage is a major yield limiting factor in rain-fed shallow lowlands. The gain in drought stress resistance breeding is low. The eastern region in the country is likely to be affected by the more drought stress. Many QTL *viz*., *qDTY1.1* (Vikram et al., 2011), *qDTY2.1* (Dixit et al., 2012), *qDTY2.2* (Dixit et al., 2014), *qDTY3.1*, *qDTY6.1* (Venuprasad et al., 2012), *qDTY8.1* (Vikram et al., 2012) *qDTY9.1A* (Dixit et al., 2012) and *qDTY12.1* (Mishra et al., 2013), *qDTY10.1* (Swamy et al., 2013), *qDTY2-2* and *qDTY3-1* (Sahmsudin et al., 2016), and *qDTY7*-1 (Sandhu et al., 2018) are detected for yield under drought stress. Six QTL viz., *qDTY1.1*, *qDTY2.1*, *qDTY2.2*, *qDTY3.1*, *qDTY3.2* and *qDTY12.1* for grain yield under drought have been transferred to flood tolerant versions of Swarna, Samba Mahsuri and IR 64 varieties (Bhandari et al., 2019). Genetic studies for several traits such as root characters, osmotic adjustment, cell membrane stability, relative water content, leaf rolling and stomatal conductance were reported for drought tolerance in rice (Venuprasad et al., 2012; Dixit et al., 2017). *Sub1* for submergence tolerance and *Xa21*, *xa13*, *xa5* and *Xa4* for bacterial blight resistance have been stacked in Swarna variety (Pradhan et al., 2019; Mohapatra et al., 2021). In addition, *Sub1*+*qDTY1.1*+*qDTY2.1*+*qDTY3.1* in CR Dhan 801, *Sub1*+ *qDTY2.1*+*qDTY3.1* in CR Dhan 802 and *Sub1*+ *qDTY2.1*+*qDTY3.1* in Sambha Mahsuri (DRR Dhan 50) are released as climate smart varieties in the country.

2.3 Climate-resilient rice breeding for semi-deep and deep-water ecologies

The ecology faces various abiotic stresses alone or in combination during the crop growth stages or in the succeeding year. The challenging problems of the ecology are submergence and intermittent water logging, drought, low plant stand, coastal salinity, and cyclonic storm. The regional problem of prolonged submergence for more than 15 days is a common feature of this ecology. The crop may face drought stress during early or terminal stage due to monsoon irregularities. Development of rice varieties with built-in tolerance to flood, water logging and drought are the basic requirement of the varieties for the ecology. In addition to *Sub1*, genes conferring more than 2 weeks submergence tolerance need to be deployed for development of submergence tolerant varieties. Purnendu, Panikekoa, Sabita, Hanseswari, Ambika, LPR 106, Borjohingia, Rayada B3, Dinesh, Nalini, Biraj and Amulya are good donor germplasms for breeding. High yielding varieties such as CR Dhan 500, Varshadhan, CR Dhan 505, CR Dhan 506, CR Dhan 507, CR Dhan 508 and CR Dhan 510 are popular in this ecology. Thailand varieties, Leuang Pratew 123 and Khao Tah Haeng 17 are good donor sources for stagnant floods, flash floods and drought stresses tolerance for breeding programs (Puckridge et al., 2000).

Direct seeding is the common practice of growing rice under rain-fed ecosystems. This system has advantages like reducing cost of cultivation, crop duration and maintenance of soil structure. Again, early direct seeding is generally practiced before the onset of monsoon as the ecology would be water logged due to rain. Therefore, high-yielding varieties with built-in anaerobic germination ability are highly needed in this rain-fed unfavorable ecology. The leveling of soil in the direct seeded fields is usually not proper. Heavy rainfall and flash floods are common in the eastern region. Water stagnates in the unleveled direct seeded fields that lead to poor germination. As a result, plant stand reduces and produces low yield. Few traditional landraces have the capacity to germinate under such anaerobic or anoxia condition. But, no popular high yielding varieties contain this character. The activity of alpha-amylase determines the germination under anaerobic condition. Five QTL for anaerobic germination (AG) *viz.*, *qAG-1-2*, *qAG-3-1*, *qAG-7-2*, *qAG-9-1* and *qAG-9-2* explaining 17.9 to 33.5% of phenotypic variance for the trait were reported in the donor variety, Khao Hlan (Angaji et al., 2010). Another six QTL identified from the landrace Ma-Zhan Red and located on chromosomes 2, 5, 6 and 7 (Septiningsih et al., 2013). The QTL, *qAG-9-2* was mapped on the chromosome 9 and located at 50 kb region in the variety, the Khao Hlan (Angaji et al., 2010). The gene encoding trehalose-6-phosphate phosphatase (OsTPP) confers the stress tolerance and has been cloned and functionally characterized (Kretzschmar et al., 2015). This QTL has also been transferred to many popular high-yielding rice varieties (Toledo et al., 2015).

Rice plant is sensitive to salinity stress both at seedling and reproductive stages of the crop. Being sensitive, rice is the preferred crop in the saline areas as the crop tolerates water-logging, and also dilutes the accumulated salts. Though the landraces are poor yielder, but the farmers of coastal areas are cultivating those due to their varying degree of salt tolerance. The presence of diversity for salt tolerance in the landraces provides opportunity for improvement of salt tolerance. Rice productivity from coastal ecology is low and unstable due to the frequent occurrence of abiotic stresses. The stresses like salinity, submergence and stagnant flooding may occur singly or in combinations which affect rice production. No high yielding variety with built-in tolerance to submergence and stagnant flooding is available. Therefore, farmers are cultivating landraces which are tolerant to both salinity and stagnant flooding. Development of rice variety with tolerance to abiotic stresses especially salinity and stagnant flooding could improve the productivity and sustainability of the coastal ecosystem. A major QTL responsible for maintaining low Na+, high K+, and Na+/ K+ homeostasis in shoots under saline condition, *Saltol* is mapped on chromosome 1 which was detected from the population of cross Pokkali and IR29. However, salinity stress is controlled by many genes. Plant breeders have transferred whole *Saltol* QTL to

many popular varieties through marker-assisted backcross breeding. However, for complete tolerance, genes controlling the terminal stage salinity tolerance need to be identified and utilized in the breeding programs. Control of salt stress through management approach is feasible but not preferred due to high cost. Thus, genetic approach of salt tolerance in rice is the promising strategy to control the stress. In coastal areas, the reality is occurrence of multiple abiotic stresses singly or in combination. Addressing multiple abiotic stress tolerance has a limited success for this unfavorable ecosystem, affected by various abiotic stresses such as salinity, submergence, water logging and even drought in some cases. Many varieties with high yield potential have been released notably *viz.*, CR Dhan 402 (Luna Sampad), CR Dhan 403 (Luna Suvarna), Panvel 3, Bhutnath, etc. but none of them is tolerant to salinity at flowering stage and have high salinity stress tolerance (>6 dS m^{-1}). Robust QTL and markers are not available for reproductive stage salinity stress tolerance for marker-assisted breeding.

Traditionally cultivated local rice varieties in coastal areas have tolerance to salinity and submergence but are low yielding. Some of the widely used varieties are: Pokkali, Chettivirippu,Vikas, Korgut, Sathi, Pichaneelu, Kuthiru, Kalundai Samba, Bhurarata, Kalarata, Karekagga, Bhaluki, Rupsal, Nona Bokra, Kamini, Talmugur, Patnai, Getu, Rahaspunjar etc. These diverse traditional rice varieties are precious genetic resources that provide ecological balance and their conservation is crucial for future food security. Using these germplasm, many high yielding varieties such as CST 1-7, Bhutnath, Panvel-3, CSR36, etc. have been developed. *Sub1* QTL has been transferred into the popular lowland rice varieties such as Bahadur, Ranjit, Varshadhan and Savitri. Now, *Saltol QTL* is being transferred into the popular varieties, namely, ADT 45, Savitri, Gayatri, MTU 1010, PR 114, Pusa 44 and Sarjoo 52 (Singh et al., 2016). The target is now to transfer *Sub1*+drought yield QTL+*Saltol* QTL into the background of popular varieties. QTL for salt tolerance at flowering stage was reported earlier based on bi-parental mapping populations, but many are not reproducible due to less trait diversity. However, genome wide association mapping exploiting large scale single nucleotide polymorphism have been practiced to capture natural variations and allelic variations of the salinity stress candidate genes. Using custom-designed array based on 6000 SNPs, 20 loci associated with Na-K homeostasis were detected (Kumar et al., 2015). They also confirmed that *Saltol* was the major salt tolerance QTL during seedling stage. In addition, they also reported the QTL for reproductive stage salinity tolerance in leaves for Na^+-K^+ ratio. New QTL were reported which are located on the chromosomes 4, 6 and 7.

Lodging of the lowland rice is mainly due to heavy panicle and by strong wind during crop maturity stage. Breaking/lodging occurs at the lower internodes of culms having thin culm diameter and poor tensile strength genotypes (Islam et

al., 2007). Pushing the resistance of the lower internode is commonly considered to measure the lodging response in rice (Berry et al., 2003). Lodging resistance is governed by many genes and the trait is complex in nature. Culm diameter and thickness are considered as main factors for lodging resistance in rice (Zhu et al., 2008; Ookawa et al., 2010). A strong correlation of culm strength with culm length, culm diameter, and yield exists in rice (Kashiwagi et al., 2008; Zhu et al., 2008). Many QTL for culm length, strength, and thickness related to lodging resistance have been identified from different mapping populations (Kashiwagi and Ishimaru, 2004; Mu et al., 2004; Kashiwagi et al., 2008; Zhu et al., 2008; Ookawa et al., 2010; Yano et al., 2015). A major QTL, *qLR1* was located at <"80 kb region on chromosome 1 for culm diameter, culm length, and breaking strength. *qLR8,* another major QTL on chromosome 8 was identified which enhanced the breaking strength in rice (Long et al., 2020). Two QTL, *SCM1* and *SCM2* were detected from *indica* rice. In addition, *SCM3* and *SCM4* QTL identified from tropical *japonica* cultivars, showed high resistance to stem-breaking and lodging (Chigira et al., 2020).

Elongation of plant parts under water is the most important target traits for QTL analysis of deep-water rice. The QTL for leaf length, internode length, and plant height increment were mapped using the population of IR74/Jalmagna cross and detected 26 QTL regulating submergence tolerance and plant elongation (Sripongpangkul et al., 2000). Using the rate of internode elongation (RIE) and lowest elongated internode (LEI), two QTL were detected on chromosomes 1 & 12, and 3 & 12 for each trait, respectively (Kawano et al., 2008). In addition, two QTL were reported on chromosomes 3 and 12 for the trait, LEI (Nemoto et al., 2004). REI, LEI, and total internode elongation length (TIL) were also mapped and three QTL were reported on the chromosomes 1, 3, and 12 in the same genomic regions by using three different populations (Hattori et al., 2007). These QTL are constantly responsible for elongation in response to flooding in deep water rice. Furthermore, the reported QTL on chromosome 12 for internode elongation in deep-water rice using near-isogenic lines (NILs) in the genetic background of Taichung65 were most effective (Hattori et al., 2008). Two novel QTL, *qTIL2* and *qTIL4* were detected for total internode elongation during the seedling stage of deep-water rice (Nagai et al., 2012). The major QTL reported by Angaji et al., 2010, Septiningsih et al., 2013 and Kretzschmar et al., 2015 for anaerobic germination need to be deployed for improvement of plant stand in semi-deep and deep-water rice.

Fe-toxicity is commonly observed in lowland rice ecology with high in soluble ferrous (Fe2+) in soil. This abiotic problem is commonly seen in the

lowlands of hilly areas. It generates reactive oxygen species (ROS) and hydroxyl radicals (OH) under the toxic situation. These compounds damage the rice plants thereby reduce grain yield. Rice plant uses various transporter genes like *OsIRT1*, *OsFRO2*, *OsVIT1*, *OsVIT2*, *OsNRAMP6*, *OsNAAT1*, *OsNAS3*, *OsNAC4*, *OsNAC5* and *OsNAC6* under toxicity condition are involved for Fe homeostasis (Pradhan et al., 2020a).

3. BREEDING FOR RESISTANCE TO PROMINENT BIOTIC STRESSES FOR CLIMATE-RESILIENT RICE

The prominent diseases and insect pests of rice observed under changed climate have been discussed. Though chemical control measures are available to control these stresses but breeding host plant resistance is preferred as it is environment friendly and cost effective. The main biotic stresses under the changed climate for upland ecology include blast (*Magnaporthe oryzae*), brown spot (*Helminthosporium oryzae*), sheath rot (*Sarocladium oryzae*), yellow stem borer (*Scirpophaga incertulas*), leaf folder (*Cnaphalocrocis medinalis*), gundhi bug (*Leptocorisa acuta*) and termite (*Odontotermes obesus*). Plant hoppers, stem borer, gall midge, leaf folder, bacterial leaf blight, sheath blight, sheath rot and false smut are the important biotic stresses faced by the lowland rice cultivators.

The brown plant hopper (BPH) is a serious sap sucking and monophagous insect. It causes heavy infestations and spreads very rapidly under high temperature and relative humidity. The hopper burn outbreak by the insect is more prominent now-a-days by wilting and drying. Though more than 40 major genes and 22 QTL for resistance have been identified but most of them are not effective against the prevalent biotypes of India. Among the resistance genes, To date, eleven resistance genes namely *Bph14*, *Bph9*, *Bph3*, *Bph6*, *Bph15*, *Bph18*, *Bph26*, *Bph29*, *Bph32, bph2* and *Bph30* have been cloned *via* map-based cloning (Shi *et al*., 2021). Few prominent germplasm lines namely Ptb 33, Manoharsali, Rasi, Ptb 10, Ptb 20, ARC333, ARC356, ARC11324 and ARC11309 are good donors against the pest. At NRRI, two highly resistant landraces, Salkathi and Dhobanumberi have been identified against the Indian biotypes and registered as unique germplasm. The recombinant lines from these two donor parents such as CR2711-114, CR 2711-76, 2711-139, CR 2711-149 and CR2712-2 are also exhibiting resistance against the pest. The white backed plant hoppers (WBPH) suck fluid from plants and cause wilting of tillers and drying of the leaves. Donor germplasms namely N22, ARC 10239, ADR 52, Rathu Heenati, AC 111, AC 1066, AC 1073, AC 124, AC 1418, IC568061, AC 111, AC 1066, AC 1073, AC 124, AC 1418 are identified for resistance breeding (Jena et al., 2018). A total of 14 resistance genes are known to control WBPH resistance in rice. The pest appears as a mixed population with BPH. The resistance genes, *Wbph7* and *Wbph8* were mapped to

the same chromosomal regions as in BPH resistance genes, *Bph14* and *Bph15*, respectively (Tan et al., 2003). Varieties like IR48, IR52, IR60, IR62 and IR 64 were reported as moderately resistant.

Among the 20 different stem borers, yellow and striped borers cause serious yield loss in rice crop. Large numbers of germplasm lines were screened for resistance against the pest at IRRI and few lines showed resistance against the pest. Screening of germplsm at NRRI, Cuttack identified germplsm lines namely TKM 6, Ratna, CO 7, CO 15, CO 21, WC 1263, NDR 402, CR 580-5, LPR 256, LPR 85, LPR 14, LPR 96-10, LPR 56-49, LPR 50, Kariawa 4, TCA 12, Bazail 65, Nali Hazara, Janaki, OR 1358-RGA-4, OR 1529-28-2, TKM 6, ARC10660, Litipiti, Daonara, Chadhei Nakhi, Dahijhil, Brahmanbojni, Mahalakshmi, Jogen, Punshi, Triveni and Saket-4 as donor parents for stem borer resistance. From the available resistance response, it seems that the resistance to the pest could be under the control of polygenic inheritance.

Rice gall midge is an important insect pest causing huge yield loss in rice production. Deployment of single gall midge resistance gene resulted in breakdown of resistance in the cultivars due to existence of more biotypes. To date, 11 resistance genes such as *Gm1 to Gm11* have been identified in rice (Zhou et al., 2020). *Gm1*, *Gm2*, *Gm4*, *Gm5*, and *Gm6* are effective against biotype 1; *Gm2* and *Gm4* against remaining 6 prevalent Indian biotypes while *Gm6* conferred resistance against four Chinese biotypes. Therefore, pyramiding of suitable resistance gene combination is recommended for broad spectrum resistance against the pest. Eswarakora, Ptb 18, Ptb 21, Siam 29, and Leuang 152 are identified as good donor germplasm materials for resistance breeding. In recent years, leaf folder of rice has also emerged as an important pest due to climate change. Chemical control approach for controlling the pest has environment concern. Control of the pest through use of resistant varieties is recommended. varieties namely GEB 24, CR 56-17, TKM6, Ptb 33, CO7, PTB33, ARC10982, Shete, Bir-Me-Fen, Kaohsiung Sen Yu 169 were reported as resistant to leaf folder (Jena et al., 2018).

Rice blast is the most serious threat for upland rice. Prevalence of new races and high mutation rate of the pathogen have resulted frequent breakdown of single resistance gene in the upland rice. Effective resistance (R) genes combinations including defense-regulator (DR) genes are required for obtaining broad spectrum resistance against the pathogen. The R genes like *Pi2, Pi5, Pi9*, *Pi21* and *Pi54* along with DR genes *Pid2, Pid3, Pid4*, *Pigm* and other effective genes in different combinations will provide a durable resistance against the pathogen. Brown spot is another serious disease commonly seen in the direct seeded rice of Bihar, Chhattisgarh, Madhya Pradesh, Odisha, Assam, Jharkhand

and West Bengal. The disease is usually more pronounced when it combines with soil moisture and nitrogen stress. Brown spot disease of rice is an important disease under climate change at relatively high temperature. Three resistance QTL, *qBS2, qBS9* and *qBS11* to brown spot disease are reported from the donor cultivar, Tadukan (Sato et al., 2008). Another resistance QTL, *BSq11* was reported from IR62266 (Katara et al., 2010). Matsumoto et al. (2017) detected a resistance QTL, *qBSR11-kc*, from the donor line 'CH45'. These QTL will be useful for marker-assisted selection for improvement of brown spot resistance in rice.

Bacterial blight is a very destructive rice disease and is observed in almost all the rice growing countries of the world. There is break down of resistance to this disease by deployment of single resistance gene due to pathogen variability. Therefore, deployment of suitable resistance gene combinations in the popular varieties is required for durable and broad-spectrum resistance in rice. A total of 45 resistance genes have been reported in rice (Pradhan et al., 2020b). Incorporation of resistance genes *xa13* and *Xa21* into popular varieties such as Pusa Basmati 1 Type 3, Taraori Basmati, Basmati 386, Jalmagna, MTU1010, Ranbir Basmati and Pusa Basmati 1509 showed resistance against the disease (Joseph et al., 2004; Rajpurohit et al., 2011; Pandey et al., 2013; Pradhan et al., 2016; Arunakumari et al., 2016; Krishnakumar and Kumarvadivel, 2018; Raina et al., 2019; Sagar et al., 2020). Varieties pyramided with *Xa21+xa13+xa5* combination showed a broad-spectrum resistance to Indian strains (Pradhan et al., 2015). This gene combination has been pyramided into the Indian popular varieties namely PR106, Samba Mahsuri (BPT5204), Pusa Basmati1, Triguna, ADT43, ASD16, Lalat, Jalmagna, Ptb39 (Jyothi), CSR30 and Safri-17 through MAB approach (Singh et al., 2001; Sundaram et al., 2008; Gopalkrishnan et al., 2008; Perumalsamy et al., 2010; Sundaram et al., 2009; Dokku et al., 2013a; Pradhan et al., 2015, 2016; Gorakhanath et al., 2017; Tanvi et al., 2018).

False smut disease is no more a minor disease due to changed rainfall, high relative humidity, temperature and N application. Two consistent QTL, *qFsr11* and *qFsr12* showed 18.0% to 19.3% phenotypic variance, respectively for false smut tolerance (Li et al., 2009). Again, Li et al. (2014) detected the consistent QTL, *qFSR11* showing phenotypic variance of 15% that is useful for breeding. Four QTL *viz.*, *qFsr6-7*, *qFsr10-5*, *qFsr10-2* and *qFsr11-2* are reported to be effective against the disease (Zhou et al., 2014). Seven varieties such as Ptb 7, Ptb 23, Ptb 24, Ptb 32, Ptb 36, Ptb 42 and Ptb 46 were free from disease when screened under field condition (Raji et al., 2016). Varieties like Harsha and Vaishak were found highly resistant; Makom, Thekkancheera, Pavizham and Karthika were resistant while Kanakom, Revathi and Prathyasha showed moderately resistance to the disease (Rashmi et al., 2016). Few QTL are reported for sheath blight resistance in

rice (Zuo et al., 2010; Zeng et al., 2015a,b; Taguchi-Shiobara, 2013), of which *qSBR11-1* located on chromosome 11 identified in *indica* rice, Tetep provides substantial resistance to sheath blight (Channamallikarjuna et al., 2010). Two wild rice species viz. *Oryza australiensis* and *O. nivara* were resistant against *R. solani*. Local varieties Biradia Bankoi, Dhusara, Ganjamgedi, Kalaketiki, Panikoili, Rajamani-K, Latamahu, Kendrapara-Kalama, Pasakathi, Tulasimali, Gangabhalu, Kandhamal-Jhalaka, KanakChampa, K-Balisara-Lakti Marchi, Laxmi, Vilash, Magra-P and Bolangir- aidipali-Mahipal were moderately resistant to the disease. Rice varieties like CR-1014, IR 64 MAS, CR Dhan 306, CR Dhan601, CR Dhan701, Kalinga-III, Satyakrishna, Gayatri, Reeta, Utkalprava, Geetanjali, Hanseswari, Binadhan 8, Varsadhan, Wita 9 were observed to be moderately resistant. Sheath rot disease causes rotting and discoloration of the leaf sheath which increases spikelet sterility. Wide spread occurrence of the disease is observed now-a-days. Germplasm lines such as Boga Jalsi, Boga Joha, Monika adhuri Joha, Tulsi Joha, Goul poriya Joha, Bokul Joha, MDR40049, CN 1035-61 and OR-090-3-158 are reported to be resistant to the disease (Jena et al., 2018).

4. FUTURE THRUST

The ongoing trend of temperature increase, erratic climate events and other stresses are posing a great challenge to higher rice production. The uncertain rainfall, temperature, CO_2 level and other greenhouse gases increase the frequency of stressful events like flood, drought, cyclones etc. are limitations to achieve food security for the increasing population. A paradigm shift in the breeding approaches must be followed for obtaining climate-smart varieties producing higher yield potential. The smart varieties should have built-in resistance to the major insect pests and diseases, should be efficient resource users and high yielder under stressful environmental conditions. In order to stake suitable genes/QTL for designing climate-smart rice, the effects of the individual QTL/gene and its combinations for synergistic effects need to be identified for achieving the target results. Search on resistance genes/QTL for the emerging diseases and insect pests for host plant resistance breeding are to be taken to reduce application of the environmental concern chemicals. Uptake of heavy metals and other toxic elements from soil to grains need to be restricted for production of healthy and safe food grain. Stress tolerant genes from unexplored wild rice need to be searched for. Handling of large population, high throughput phenotyping and genotyping and data management tools will be the key steps in future rice breeding.

The rice breeders need to be updated to handle stacking of multiple genes for various abiotic and biotic stress tolerance genes/QTL including yield in the coming days. Breeding techniques by integration of biotechnology with conventional breeding strategies will be employed to breed smart rice varieties.

The new tool kits from biotechnology have potential to address the challenges of developing stress tolerant smart-rice varieties.

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Hybrid rice research and imminent prospects

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SUMMARY

The development of hybrids for enhanced productivity per unit area is one of the greatest discoveries in the history of crop breeding. It is a practically feasible and readily adoptable to enhance the productivity. Given to its remunerative yield, rice hybrids are commercialized in over 40 countries which create huge business opportunities. After China, India has made commendable progress and released a total of 127 *indica* hybrids which is grown in ~3.5 mha (8.0% of total rice) area. Moreover, several heterotic parental lines (CMS and restorers) have been developed indigenously under diverse genetic and cytoplasmic background. ICAR-National Rice Research Institute has been pioneering to develop this technology, commercialized three hybrids *viz.,* Ajay, Rajalaxmi and CR Dhan 701 for irrigated-shallow lowland ecosystem. Biotechnological interventions have augmented in excavating and deployment of desirable genomic regions enhancing genetic gain for yield, quality and sustainability in hybrids. Furthermore, hybrid rice (HR) technology has opportunity for additional job to over 20.0 million rurals and income (70% more than production cost) to the farming community. Thus, it has great scope for improvement of livelihood of the poor farmers of the nation.

Keywords: Hybrid rice**,** enhanced productivity, diverse genetic, heterotic, cytoplasmic background

1. INTRODUCTION

Heterosis is the superiority of F_1s over either parent apprehends ample hybrid-vigour in crop plants. It has helped the farming communities and fascinated geneticists for breeding excellence over centuries (Verma et al., 2018). Suitable allelic combination has made yield advantage in hybrid than HYVs. Consummation of allelic as well as content variation and expression patterns of genes is found to be substantial contributor towards heterosis development (Schnable et al., 2013). It has been instrumental for considerable genetic gain in crop plants, applied in various ways before early scientific elucidation made by Darwin in maize. Heterosis in rice was first reported by Jonse in 1926 but realized commercially after seed production system was developed during 1970s in China. The renowned rice breeder, Yuan Long Ping has led the hybrid rice (HR) breeding in China with a vision to make this technology possible to be commercially viable.

Subsequently, Li (1970) discovered a natural male sterile (MS) mutant in *indica* rice and pollen abortive genotypes from wild rice (*Oryza rufipogon)*. Later on, *Oryza rufipogon* was found most suitable and utilized as donor of MS for development of cytoplasmic male sterile (CMS) lines. Consequently, several good combining wide abortive (WA) *indica* CMS lines like Erjiunan1A, Zhenshan 97A and V20A; and restorers *viz*., Taiyin1, IR4 and IR1 were developed and utilized in rice hybrid varietal development. After about one decade of rigorous research efforts, world's first commercial rice hybrid Nanyou 2 was released during 1974 for cultivation in China. Later in 1987, relatively more heterotic (>20% heterosis) two-line based seed system was developed which yielded super rice hybrids (1996) that supplemented immensely towards Chinese food security and livelihood.

Encouraged with the success of Chinese hybrid rice programme, several countries including India have adopted HR technology. In India, it was started in1989 when Indian Council of Agricultural Research (ICAR) launched a focused and time bound project 'Promotion of Research and Development efforts on Hybrids in Selected Crops' at 12 centres. Subsequently, country's first rice hybrid APRH-1 was released by APRRI, Maruteru for Andhra Pradesh during 1994 and India became the 2nd country after China to commercialize hybrid rice. So far, India has commercialized a total of 127 rice hybrids (38 from public and 89 from private sector) suitable for diverse ecologies (115-150 days) which covers ~3.5mha of country's total rice acreage.

This technology is able to enhance farm productivity up to 15-30% (Rout et al., 2020; Luo et al., 2013). Given to its remunerative values, this technology has been adopted and commercialized in over 40 countries which create huge business opportunities. Further, it provides extra income (Rs. 80000-85000/ha net return) and additional employment opportunity to the 20 million households (requires 100-105 extra man days/ha area of HR seed production) (Rout et al., 2020). However, owing to some inherent limitations like inefficient seed system (CGMS based three-lines), meagre extant of heterosis (10-15%) and poor grain quality in the hybrids, this venture could not be adopted much as anticipated initially. Problems like instability and non-abundancy of MS coupled with CMS load, low seed producibility in CMS, poor grain and sustainability under biotic and abiotic stresses and hybrid sterility in *indica/japonica* hybrids etc. are the major limitations. This article is aimed at review the research progresses made and devise the future breeding strategies for further betterment of this technology.

2. BREEDING COMPONENT AND SYSTEM IN HYBRID RICE DEVELOPMENT

The HR seed production needs some parental specificity like complete and stable male sterility and substantial out-crossing ability in seed parents which excludes

manual emasculation and facilitates commercial seed production. The naturally occurred MS systems (CMS, CGMS and GMS) or genetic tools make this endeavour be realized in rice are discussed in following heads

2.1. Male sterile system

The MS is crucial genetic tool for HR breeding as it excludes most cumbersome, time taking and expansive activity *i.e.,* manual emasculation (Verma et al., 2018). It is the condition where anthers impair to dehisce and produce viable pollen thus encourage allogamy or open pollination. In plant, MS was first time reported by J. G. Kolreuter during 1763 which later on discovered in over 600 plant species. Primary evidence of MS in rice was seen in reciprocal crosses of *indica/japonica* at ICAR-National Rice Research Institute (Sampath and Mohanthy, 1954). In rice, MS is accustomed either by mitochondrial gene in association with nuclear genes (CMS) or nuclear genes alone (GMS) which led anomalous changes in sporogenous tissue (sporophytic or gametophytic tissue) and MS (Chen et al., 2014). In the sporophytic MS, sporogenous tissues like tapetal and meiocytes which are nourishing developing microspore are impaired due to reduced ATPase activity and cause pollen abortion. However, microspore and pollen development get impaired in gametophytic MS. To date, naturally two types of MS systems namely, cytoplasmic male sterile (CMS) and genetic male sterile (GMS) are discovered.

The CMS is maternal/uniparental hereditary trait instigated by chimeric construct of the mitochondrial genome (Table 3) having diversified mechanism of MS expression. The wild abortive CMS (WA-CMS) which is more stable and has restorer abundancy is utilized extensively in hybrid development is sporophytic in nature. Sterility in WA-CMS is caused by a constitutive gene WA352c located downstream of *rpl5* (comprised four segments, *orf284, orf224*, *orf288* and *cs4-cs6*) encodes a 352-residue putative trans-membrane protein. The translates of WA352c inhibits nuclear-encoded *COX11* (essential for the assembly of cytochrome c oxidase, TCA) which arrests ATPase activity in tapetal tissue and pollen abortion (Tang et al., 2017). The BT-CMS in contrast, is gametophytic in nature where dicistronic mitochondrial chimera, *B-atp6-orf79* which encodes a cytotoxic trans-membrane peptide *ORF79,* accumulates specially in microspore and causes male sterility (Wang et al., 2006). The *orf79* interact with P61and abate the activity of mitochondrial complex III which elevate oxidative stress and causing MS. Likewise, in HL-CMS transcript of *orfH79* gene interacts with mitochondrial ETC complex III (P61) and led MS due to reduced ATPase (Wang et al., 2013). However, MS in CW-CMS which has anther-specific mitochondrial retrograde regulation for nuclear gene expression is caused by mitochondrial *orf307*. This is a gametophytic male CMS where pollen grains appear normal but unable to germinate.

The GMS on the other hand is instigated by nuclear recessive genes are widely distributed and ideal for hybrid rice breeding but lack of effective ways to propagate the pure GMS lines at commercial scale make it impossible to use for hybrid rice production. Despite, several GMS lines available having threshold nature of MS expression, requires specific climatic regime hence called environment sensitive genetic male sterility (EGMS). EGMS in which MS occurs under elevated temperature i.e., >30^{0}C is called temperature sensitive genetic male sterile (TGMS) whereas, if it occurs under long day regime i.e., >13.5 hrs is called photoperiod-sensitive genetic male sterility (PGMS). This system is unique to be utilized in two-line hybrid rice breeding which excludes requirement of maintainer and comparatively more heterotic (5-10%) than three-line hybrids. In addition, rice line, Pei'ai 64S is discovered with combined nature (PGMS and TGMS) of MS expression, is referred to as P/TGMS. The PGMS- Nongken 58S (NK58S) and TGMS - Annong S-1 and Zhu1S and their derivatives are extensively (>95% two-line hybrid) utilized in the Chinese hybrid rice breeding programmes. Recently, a unique EGMS system (*csa*-carban starved anther mutant) is identified where MS is occurring under short day regime (<12.5 hrs) is called reverse PGMS (rPGMS) (Zhang et al., 2013). This system has great scope in development of two-line hybrids for tropics and subtropics (Rout et al., 2020).

In order to vitalize HR seed production system, genetically engineered/ edited MS lines having restorer abundancy and 5-10% more heterosis ability were developed. The *indica* rice maintainer line, M2B was transformed into sterile, M2BS with partial-length HcPDIL5-2a (*Hibiscus cannabinus* protein disulphide isomerase-like) genetic construct. Besides, a MS is created by combining cysteine-protease gene (BnCysP1) of *Brassica napus* with anther-specific promoter (P12, promoter region of *Os12bglu38* gene) which is restored by genetically engineered line carrying BnCysP1Si gene silencing system (Rao et al., 2018). Zhou and co-workers (2016) have edited TGMS gene *tms5*through CRISPR/Cas9and developed 11 "transgene clean" MS lines.

2.2. Fertility restorer system and mechanism

The pollen parents help restore fertility in CMS is called restorer, generally governed by mono or oligo nuclear loci with diversified range of functional specificity (Verma et al., 2020; Katara et al., 2018). In rice, total ten *Rf* genes (*Rf1a, Rf1b, Rf2, Rf3, Rf4, Rf5, Rf6* and *Rf17, Rf98 and Rf102)* have been discovered, amongst seven (*Rf1a, Rf1b, Rf2, Rf4, Rf5*, *rf17* and *Rf98)* are functionally characterized. Majority of the *Rf* loci in rice are dominant in nature (except *rf17*which restore CW-CMS) can restore fertility in heterozygous state. The genes *Rf1a* and *Rf1b* (Chr.-10) restores fertility in BT-CMS, encodes pentatricopeptide-repeat (PPR) rich protein which degrades transcripts of mitochondrial *atp6–orf79* and restores fertility in derivative

hybrids (Wang et al., 2006). Whereas, *Rf5 or Rf6* genes restore fertility in HL-CMS. The gene, *Rf5* encodes PPR family protein 'PPR791' which bind with GRP162 (glycine rich protein) and transcripts of *atp6-orfH79* and neutralize sterility by cleaving aberrant transcript of *atp6-orfH79* at 1169 nucleotides position (Hu et al., 2012). The *Rf6* on the other hand produces a novel PPR protein (duplicate PPR motif 3-5) which along with hexokinase (*osHXK6*) targets defective transcript of *atp6-orfH79* at 1238 nucleotide position and restore fertility in HL-CMS (Huang et al., 2015). The gene *Rf3* and *Rf4* (Chr. -1 and 10, respectively) encodes a pentatricopeptide protein (PPR) where RF4 cleave the abnormal transcript of *WA352* and RF3 suppress translation of *WA352* and thus, restoring fertility in WA-CMS. Fertility in LD-CMS is found to be restored by two genes, *Rf1* or *Rf2*.The *Rf2* encodes a glycine-rich protein in mitochondria and restores fertility. Moreover, CW-CMS is restored by a single recessive gene (*rf17)* which is a retrograde-regulated male sterility (*rms*) gene (Toriyama et al., 2016) (Table 4).

2.3. Hybrid rice breeding system

In rice, natural out-crossing is very low (ranged only 0.3-3.0 %), thus, commercial HR seed production is a cumbersome and expensive task, needs specific parental lines and best agro-management practices (NRRI Technology bulletin-114). The discovery of MS system makes this technology realized commercially in rice. Based on male sterility mechanism and threshold nature of expression (TGMS, PGMS, P/TGMS, rPGMS) and number of parental lines involved, three types of HR seed production systems, namely CGMS based three-line system (involves A, B and R lines), two-line system (only two parents, A and R) and one-line system (apomictic based, still in budding stage) are available (Rout et al., 2020). Amongst, CMS based three-line system is extensively utilized (>90% of world's hybrids) in HR production.

2.3.1. Three-line HR system

Three-line system involves three parental lines (A-line or cytoplasmic male sterile), B-line (maintainer) and R (restorer) and two steps in seed production, i.e., CMS maintenance and HR seed production. In this system, CMS or A-line because of its eliminated need of emasculation served as seed parent in large scale seed production. Whereas, the B-line which is fertile isogenic to CMS, differs for only sterility trait and utilized as pollen parent to maintain male sterility in CMS. In contrast, R-line is genetically diverse parent, having ability to restore fertility in CMS based hybrids, thus, utilized as pollen parent in HR production.

A suitable CMS line should have complete and stable male sterility, ample out-crossing ability, wide compatibility and least CMS/genetic load. The seed parent with heavy panicle, low test weight (1000 grain weight) and narrow, semi-erect leaf configuration assures more seed production and low seed rate. In

India, generally HR seed production is keen to Rabi season, hence, seed parent should have seedling stage cold tolerance and flowering stage heat tolerance. Whereas, restorer (R-line) should have substantial genetic distance with CMS/B-lines which is essential parameter to determine magnitude of heterosis in hybrids (more diversity more heterosis and *vice -versa*). Besides, R line must have high breeding values with strong fertility restoration ability, ideal plant type, better grain quality, considerable source-sink balance and enhanced sustainability against biotic and abiotic stresses.

2.3.2. Two-line HR system

This HR system has eliminated need of maintainer for propagation of MS line, thus simple and more efficient for seed production, involves two parents *i.e.,* A-line and R-line. It is considered to be an effective alternative to CMS system, has specific merit as entails only one step for HR production. In this system, any compatible genotype irrespective of their fertility restoration ability can be utilized as pollen parent. EGMS system is comparatively more heterotic (5-10%) as it does not exert any genetic illness on the carrier plant. The male sterility in EGMS lines is governed by major mono or oligo genes, thus easily transferable to any genetic background. Because of its rejecting needs for specific restorer genes in the pollen parents, found ideal for harnessing heteroses is in inter-subspecific (*indica/japonica*) pool.

2.3.3 Apomixis based one-line HR

This system is built on apomixis (Apart from mixing) phenomenon (embryo development without fertilization of gametes). In this strategy, HR seeds once generated need not be produced further, here hybridity/heterozygosity/ heterosis will be maintained as of in original through clonal production. In rice, this system is still in nascent stage, recently, Delphine et al., 2016 reported three mutated genes i.e., *SPO11-1, REC8* and *OSD1* in *Arabidopsis thaliana* which are able to turn meiosis into mitosis, nourishing female gametophyte to their development and helps in production of clonal male and female gametes. Crossing a *MiMe* plant as male or female with a line whose genome is eliminated following fertilization (lines expressing modified CENH3) leads to the production of clonal offspring (Mieul et al., 2016). Recently, a strategy based on the advance technique i.e., CRISPR/Cas 9 has been utilised to introduce apomixis into rice (*Oryza sativa*) by mutating the three combined genes *OsSPO11-1, OsREC8*, *OsOSD1*, and *OsMATL* to get a *MiMe* phenotype (Xie et al., 2019).

3. HYBRID RICE RESEARCH AND DEVELOPMENTAL PROGRESS

3.1. International status

The hybrid rice technology is considered to be one of the paramount innovations in the modern time, contributed greatly in enhancement of yield and other

parameters in several crops. The Chinese scientists have been pioneering in exploitation of commercial realization of heterosis, developed requisite parentage and package of practices and released world's first hybrid for cultivation during 1974. At beginning, WA-CMS Zhenshan 97A was extensively utilized for HR development. Subsequently, several CMS with alternative MS mechanism were discovered, characterized and utilized in HR breeding. Initially, the low seed producibility in CMS was a concern for HR commercialization which later on rationalized through research and management efforts.

The government of China has supported this technology in pilot mode and established HR seed businesses during late1970s at all level, comprehensively. They have supported intensive mechanization for HR seed production which helped in rationalizing the seed production field plan (from 2:6-8 planting row-ratio to 2: 40–80). Consequently, Chinese could achieve substantial seed producibility up to 2.7–3.0 t/ha which was further improved to 3.4t/ha and maximized HR acreage.

Over past three decades hundreds of HR varieties were developed which covers over 60% of the total rice area (30 mha) and contributed Chinese national food security, substantially. Further, the Ministry of Agriculture (MOA), China has launched super hybrid rice project during 1996 which resulted total 73 hybrids (52 three-line and 21 two-line). Super hybrid P64S/E32 released recently has recorded new height of yield potential of 17.1 t/ha with some striking characteristics (Yuan et al., 2017).

Along with China, this technology is also adopted in over 40 countries across the world. International Rice research Institute (IRRI) rejuvenated its interest in the prospects of hybrid rice technology in 1979 after China demonstrated its debut of hybrid rice cultivation. Collaboration between the Food and Agriculture Organization (FAO) with IRRI, Japan, China and some selected national agricultural research centres (Indonesia, India, Brazil, Colombia, Vietnam, People's Republic of Korea, and France) promoted hybrid rice research and development with emphasis on the rapid and quick transfer of many major innovation in hybrid rice technologies. Collaborative work between IRRI and PhilRice was initiated in 1988, with parental line and experimental hybrid evaluation as the major areas of collaboration. In 1989 the first rice hybrid "IR64616H" was released in Philippines and registered as "PSB Rc 26H" and named as "Magat" hybrid in 1994. Area under hybrid rice cultivation was started with very small area at 5 000 hectares in 2001 and spiked to 369000 hectares in 2005. IRRI scientists developing hybrids using conventional and molecular marker technologies with yield and yield heterosis, bacterial blight resistance, submergence tolerance, drought tolerance, low chalk and high out-crossing ability (Xie, 2011). To

strengthen the collaboration between the public and private sectors and to enhance the dissemination of hybrid rice technology, Hybrid Rice Development Consortium (HRDC) started at IRRI in 2008 with 39 public and private organizations.

Initially, International Rice Research Institute (IRRI), Philippines has supported this programme technically and supplied requisite genetic materials to rest of the world, and thus, most of the rice growing countries have established their own HR breeding programmes and contributed several heterotic hybrids. India is the 2nd country after China who has adopted HR technology in plan mode during 1989 and made substantial progress. Till today, total 127 heterotic hybrids for diversified rice ecologies has been developed which covers ~3.5m/ha rice area in India. Afterward, Vietnam has HR technology in 1992 and developed hybrids with average productivity of 6.3–6.8 t/ha which covers 10% of their rice area. Philippines has introduced HR in 1993 and commercialized popular hybrids like Magat, Mestizo, Mestizo 2, Mestizo 3, Bigante, Magilla, SL8H, Rizalina 28 etc. HR seed production in Philippines has been picked up by 'seed growers' cooperatives who are producing around 60-70% of them. Traditional rice growing country Bangladesh has adopted several heterotic hybrids from China, India and Philippines which covers ~ 800,000.0 ha area. Indonesia has also developed popular hybrids like Hipa7, Hipa 8, Hipa9, Hipa10, Hipa11, Hipa12 SBU, Hipa13, Hipa14 SBU, Hipa Jatim1, Hipa Jatim2 and Hipa Jatim3 which are being commercialized extensively with additional yielding ability of 0.7-1.5 tons/ha over the respective duration inbreeds under low land ecosystem.

USA is the latest introducer of this technology during 2000 and commercialized several rice hybrids, majority of those are based on Clearfield (CL) technology which offers selective management of weedy form of red rice. The hybrids viz. Clearfield-XL729, Clearfield-XL745, Clearfield-XP756 (late-maturing) and Clearfield-XP4534 (ideal plant type) have shown yield superiority ~16-39% over inbreeds.

3.2. National status

India has started research on hybrid in rice during 1989 and released first hybrid by SVRC, Andhra Pradesh during 1993-94. So, far, country has released a total of 127 (*indica/indica*) hybrids with 15-20% yield superiority for diverse agro-climatic conditions (115-150 days). Recently, Savannah Private Limited has developed two-line rice hybrids which are commercialized as SAVA-124and SAVA-134. In addition,>100 CMS lines under diversified genetic and cytoplasmic background are developed, amongst, the promising CMS like CRMS 8A, CRMS 31A, CRMS 32A, CRMS 53A, CRMS 54A, CRMS 56A, PMS10A, DR8A, PMS 17A, APMS 6A, PUSA 5A, RTN 12A, PUSA6A, etc. are utilized substantially in India and abroad. Remarkably, a medium duration CMS, CRMS 32A having seedling stage

cold tolerance, developed indigenously at NRRI, India under Kalinga-I cytoplasm is found suitable for *boro* ecology, Hybrids Rajalaxmi and KRH 4 which are popular among rice growers are developed utilizing CRMS 32A (Kalinga-1).

At beginning, Indian HR breeding program was technically supported by the IRRI, Philippines and Food and Agriculture Organization (FAO), Rome; and financially supported by United Nations Development Programme (UNDP), Mahyco Research Foundation, World Bank funded National Agricultural Technology Project (NATP) and IRRI/ADB etc. Union government of India on the other hand supported scaling up and promotion of HR through various schemes, RKVY, BGREI etc. This technology is further sustained by ICAR through platform research project 'ICAR - Consortium Research Platform on Hybrid Crops Hybrid Technology for Higher Productivity in Selected Field and Horticultural Crops, at the 09 research institutions in the country. Indo-ASEAN agency is also funded substantially for exploitation of more heterotic inter-subspecific genetic pool for further breeding excellence in HR.

The hybrids released in India are adaptable to tropical and subtropical ecologies, having unambiguous specificity towards habitat, sustainability and consumer preferences (Table 1). Therefore, to harness maximum potential and benefit, only suitable hybrids should be adopted under recommended condition/ area.

Three-line hybrid is MS carrier found very prone to elevated temperature which is deleterious for seed development. Hybrids like CRHR 105, CRHR 106, CRHR 150, 25P25, 27P31 are heat tolerant in nature, therefore, suitable for yield enhancement under heat stress. The hybrids, JKRH 401, US 382, US 312, Indam

Table1. Suitability of rice hybrids againstbiotic and abiotic stresses

S. No.	Stress	Hybrid
1	Rain-fed upland	DRRH-2, Pant Sankar Dhan-1, Pant Sankar Dhan-3, KJTRH-4
2	Saline soil	DRRH-28, Pant Sankar Dhan-3, KRH-2, HRI-148, JRH-8, PHB-71, Rajalaxmi
3	Alkaline soil	Suruchi, PHB-71, JKRH-2000, CRHR-5, DRRH-2, DRRH-44, Rajalaxmi
4	*Boro* season	Rajalaxmi, CRHR-4, CRHR-32, NPH 924-1, PA 6444, Sahyadri, KRH 2
5	BB resistant	BS 6444G, Arize Prima, Rajalaxmi, Ajay, CR Dhan 701, PRH 10
6	BPH resistant	Arize AZ 8433 DT

200-17, DRRH3 are N use efficient, so, appropriate for the soil which is poor in N content. Moreover, hybrids RH 1531, Arize Tej, CRHR 105, CRHR 106 and PNPH 24 are mid-early maturing varieties suitable for water scarcity situation. The coastal and shallow-lowland areas which is sharing ~32% of the total rice area but remains low productive, can be benefited by adopting late maturity HR varieties, CRHR 32, Arize Dhani, CRHR 34, CRHR 102, CRHR 103 and Sahyadri 5 (Table 2).

Table 2. Hybrids suitable for specific condition/quality

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

3.3. NRRI's contribution

The ICAR-National Rice Research Institute (NRRI) has been pioneering to start HR breeding in late of seventies of 20thcentury, quite before the project mode project launched during 1989 by ICAR. The institute has acquired requisite genetic materials (CMS *viz*. V 20A, Yar Ai Zhao A, Wu10A, MS 577A, *Pankhari* 203A, V 41A, Er-Jiu nanA; respective B-lines, and 9 other maintainers; and 13 good restorers) from IRRI (NRRI annual report 1981-82). The target oriented inter-disciplinary approach was adopted which could fulfil the objectives to develop parental stock (A, B and R-lines) of hybrids for irrigated and shallow-submergence ecosystems. The mid-late duration (140-150 days) hybrids are more suitable for the rainfed shallow-lowland area. Keeping in views, institute has developed three rice hybrids, namely, Ajay, Rajalaxmi and CR Dhan 701 suitable for this ecosystem. The institute has also developed several stable CMS lines (WA, Kalinga-I and *O. perennis* etc. MS cytoplasm), maintainers and good restorers for further HR breeding invigoration. Total 57 CMS lines under diverse genetic and cytoplasmic background are developed, amongst, Annada A (WA), Pusa 33A (WA), Sarasa A, Manipuri A (WA), Kiran A (WA), Moti A (WA), Deepa A (WA), Krishna A *(O. perennis),* Krishna A (Kalinga I), Padmini A, Mirai (Kalinga I), PS92A and Sahbhagi dhan A etc. are more prominent and extensively utilized for hybrid

development. Besides, medium duration CRMS31A (WA) and CRMS32A (Kalinga-I) are extensively utilized for HR development in India and abroad. The medium late duration CMS, CRMS24A and CRMS40A developed under nucleus Moti and Padmini are suitable for breeding late duration hybrids. Further, short duration CMS, CRMS8A, CRMS51A, CRMS52A and CRMS53A which are able to sustain drought stress are also being utilized for hybridization of rainfed upland ecosystem.

The late duration hybrid, CR Dhan 701 (IET 20852) is commercialized for irrigated and shallow lowland area of Bihar, Gujarat and Odisha. This is a MS grain hybrid with > 60% HRR and 7.5 t/ha yield's ability, sustainable under low-light area, therefore, having great scope for the states of eastern part of India where low-light during wet season limits potential expression of hybrids. In addition, hybrid Rajalaxmi (125-130 days) is released through SVRC 2006/CVRC 2010 for irrigated and shallow-lowland area of Odisha and *boro* area of Assam and Odisha. It has seedling stage cold tolerance, hence, suitable for *boro* area. Ajay (CRHR7), a medium duration hybrid bearing long slender (LS) grains is released for irrigated and shallow-lowland area of Odisha. Given to their remuneration ability and popularity in eastern states, altogether 19 private seed agencies have taken license for commercialization.

Genomics based trait development/genetic diversification strategy is adapted to enhancing genetic gain for yield, quality and sustainability of hybrids (Dash et al., 2016). The parents of hybrids, Ajay, Rajalaxmi and CR Dhan 701 have been improved for resistance against most devastating rice disease of rice, bacterial blight (Dash et al., 2016). The submergence and salinity are the two main abiotic stresses occurring frequently in the rain-fed shallow lowland area, and cause significant yield reduction in rice. Therefore, to patch-up the problems and make HR more sustainable, institute has stacked submergence and salinity tolerant QTL in the CMS, CRMS31A/B and CRMS32A/B. Low seed producibility in Indian CMS still remain as bottleneck in expanding HR area in India. The institute is working on this aspect, introgressed long stigma exertion traits from *O. Longistaminata* into CRMS31A and CRMS32A. Despite huge technological progress and interventions, factor(s) responding heterosis in rice still remain elusive. To unravel the insights, transcriptomic analysis of hybrids, Rajalaxmi and Ajay are accomplished and interpreted (Katara et al., 2020). Parental line specificity for fertility restoration in CMS based hybrids is one of the major limitations in HR breeding as availability of restorers is very scarce in nature (only 15% of rice genotypes are restores) (Katara et al., 2017). Therefore, institute has taken initiatives to address the issues by introgression fertility restorer gene(s), *Rf3* & *Rf4* in partial restorer (PR) but good combiner genotypes. PR lines, Mahalaxmi and Gayatri were stacked with restorer gene(s) *Rf3* & *Rf4* through MABB. Further,

to address the IP issues and ensure pure seed availability to the stakeholders, hybrid specific signature markers are developed which could distinguish altogether 32 hybrids (Verma et al., 2018).

4. POTENTIAL OMICS INTERVENTIONS IN HR

Biotechnological advancement has opened remarkable opportunities to the breeder and breeding *per se* in enhancing their efficiency and accelerated varietal development. It helped HR in various ways like mining desirable traits and their incorporation, molecular characterization etc. Varietal identity/purity of hybrids and parents is mandatory to assure the proprietorship (IPR issue) and pure seeds to the stakeholders, usually performed through Grow-Out-Test (GOT) which is very expensive and time consuming (takes one full rice season). Genomic tools in this regard established a impartial and appropriate alternate for GOT. Several DNA-based co-dominant markers are available for genetic purity analysis in hybrids/parents (Behera et al., 2012; Verma et al., 2017). Besides, genomics-assisted selection/MABB/genome editing tools are providing strong utensils for improvement of trait of interest in crop plants. Rice hybrids, Rajalaxmi, Ajay (Dash et al., 2016), PRH 10, BS 6444G (Basavaraj et al., 2009), Guangzhan63-4S, Shanyou 63 (Zhang et al., 2006; Huang et al., 2012); and parents of CR Dhan 701, restorers of Minghui 63 and Mianhui 725 (Dash et al., 2016; Chen et al., 2014), Zhonghui218 and Zhonghui8006 (Cao et al., 2005) etc. has been improved for bacterial blight (BB) resistance. The blast and BB resistant genes are also successfully stacked in popular CMS, Rongfeng A (Fu et al., 2012), Pusa 6A (Singh et al., 2015), RGD-7S and RGD-8S (Liu et al., 2008). Besides, sustainability of hybrids under abiotic stress like salinity and submergence are improved by deploying tolerant QTL in CMS, CRMS 31A and CRMS 32A (NRRI newsletter 2015). The quality of HR which is major concern is improved by stacking QTL/genes for quality traits in parents, Zhenshan 97A (Zhou et al., 2003), Xieyou57 (Ni et al., 2011). The yield related QTL *yld1.1* and *yld2.1* were introgressed in restorer 'Ce64'from *O. rufipogon*to (Duan et al., 2013). Hybrid sterility (HS) in inter-subspecific (*indica/japonica*) hybrids successfully addressed through genome editing tool 'CRISPR/Cas9' (Shen et al., 2017).

Selection of heritably compatible, well combining parents needs test cross evaluation which is tedious and time-consuming process. Advance genomic tools are very useful for selection of most compatible parent with great accuracy at early crop stage, facilitates speedy HR development. Plenty of DNA markers (linked organic markers) are available, amongst, RM6100 and DRRM Rf3-10 of restorer gene(s) *Rf4* and *Rf3*respectivelyare utilized for assessment of fertility restoration efficacy of pollen parents; S5 InDel for wide compatibility analysis (Katara et al., 2017; Priyadarshi et al., 2017; Revathi et al., 2020).

Hybrid sterility (HS) is common annoyance threatening inter-subspecific hybrids (5-10% more heterosis), caused by the genes at *Sb*, *Sc*, *Sd* and *Se* (Guo et al., 2016) and the gene at *S5* locus. Presence of functional genic region in one parent confers complete fertility in resulting hybrids. These genes can be assessed and stacked by employing co-segregating markers (S5-InDel, genic marker to $S5^n$ (Priyadarshi et al., 2017) and G02-14827 (genic marker) PSM8, PSM12 and PSM180 (linked SSR); IND19 and ID5 (indel markers) to *Sb*, *Sc*, *Sd* and *Se*, loci).

Extent of genetic diversity among parents and their breeding values are key determinant of heterosis in hybrids. There are plentiful STMS, SNP markers are available offered selection of genetically diverse parents carrying maximum breeding values *per se* substantial heterosis or genetic gain in hybrids. Consequently, several heterotic groups have been developed and utilized for three-line and two-line hybrid development in rice (Lu and Xu, 2010).

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

5. INSIGHTS INTO HETEROSIS IN RICE

Harnessing heterosis is most suitable strategy to enhance genetic gain for yield (Wang et al., 2005). The genetic basis of heterosis is extensively studied and explained with several models (Zhou et al., 2012). Several research insights support that dominance and single-locus over-dominance of responding alleles are key contributor of heterosis in rice (Zhou et al., 2012; Li et al., 2015) but details of genes/genomic region and mechanism working still remain elusive. In most of the cases, heterosis related loci shows partial dominance for yield related traits, whereas, better-parent heterosis for overall traits (Huang et al., 2016). The gene, heading date 3a (*Hd3a*) considered as key for heterosis in *indica* hybrids control both grain yield and flowering is acting through incomplete dominance

Table 3. Cytoplasmic diversity in rice CMS.

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

CMS group	Associated ORF	Protein	Cytoplasm source	Representative CMSline
NX-CMS	UK	UK	Selected from F2 male sterile plants in the progeny of Wanhui 88 (*indica*) x Neihui 92–4(*indica*) nucleus	Neixiang 2A, Neixiang5A
Y-CMS	UK	UK	Yegong (*indica* landrace) cytoplasm	Y Huanong A
CW-CMS (G)	*orf307*	Mitochondrial protein	*Oryza rufipogon* Griff.	IR24A, IR64A
2. Environment sensitive genetic male sterility (EGMS)				
PGMS	*pms3*	Noncoding RNA	Nongken 58S, PGMS mutant of *japonica* cultivar Nongken 58	7001S, N5088S
P/TGMS	*p/tms12-1*	noncoding RNA	Photoperiod and temperature sensitive genic male sterile (P/TGMS) derived from Nongken 58S	Pei'ai 64S
TGMS	*tms5*, *RNase* Z^{S1} (loss in function)	Nuclease enzyme	Spontaneous TGMS mutants of Annong S-1 and Zhu 1S	Guangzhan 63S5, Xinan S
rPGMS	*csa* OsMST8	MYB transcript regulator	Carbon starved anther *(csa)* mutant of *japonica* cultivar 9522	9522S

under heterozygosity (Kojima et al., 2002). Utilizing these findings, breeders will be able to design their strategies for further excel the HR breeding. The fact of heterotic QTL/ genes can also help to improve grain quality in HR. For instance, *waxy* gene governing amylose content and chalkiness grain is tightly linked to *Hd3a* on Chr.-6, thus, through genomics intervention both quality and heterosis can be enhanced, simultaneously. However, rice breeders are still in dilemma that what is the exact reason for the over-dominance phenomenon or why the dominant effects are more frequent than the recessive effects in rice.

6. CHALLENGES AND POTENTIAL RESEARCHABLE ISSUE

6.1. Challenges

The HR researchers are severely challenged in their efforts to secure high levels of heterosis in rice. China's impressive levels of heterosis are harnessed in the hybrids adoptive to temperate-region. Whereas, in India for tropical ecosystem which is low productive (10–15 %, marginal heterosis). Thus, exploration and exploitation of inter-subspecific (*indica/t. japonica*) hybrids which are more heterotic (20-25%) than *indica* hybrids, is a workable strategy to further enhance the genetic gain in rice hybrids. Two-lines and inter-subspecific (*indica/japonica*) hybrids which are comparatively more heterotic (5-10%) than three-line *indica* hybrids, however, owing to some inherent limitations, these are yet to be exploited in India. Inevitable difficulties in seed production of two-line hybrids and poor grain, eating quality and hybrid sterility (HS) in inter-subspecific hybrids, both could not be exploited in the countries like India who has vast climatic diversity and food affection. HR breeding in India still depending on cytoplasmic-genetic male sterile system which is comparatively less heterotic (5-10%). Besides, poor grain quality of rice hybrids is a concern to the farmers in high-productivity irrigated areas, who produce marketable surpluses. The key issue cantered around is amylose, the starch molecule that gives milled rice its specific appearance and character after cooking. In hybrids, consumable parts are F_2 grains, segregating for various quality traits, hence very poor in quality.

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

Outside China, WA-CMS or their lineage are commonly utilized as seed parent in > 90% rice hybrids. Though, several alternative MS source, BT-CMS, HLCMS, CW-CMS are identified in China, but non-accessibility of those, other

countries relied only on WA-CMS which has several intrinsic aberrations like slow growth, partial panicle exertion etc. CMS systems are mainly restricted by the narrow restorer resources that make it difficult to breed superior hybrids. These narrowed genetics of sterile cytoplasm limits the extent heterosis exploitation and make hybrids vulnerable to many biotic and abiotic stresses. Hence genetic diversification/ improvement of seed parents (CMS lines) which is most neglected research activity particularly in India, with focused goal is imperative to sustain this technology further.

Low seed producibility (1.5-2.5 t/ha) in the CMS available creates another nuisance, restricts seed abundancy and area expansion in India. Therefore, trait development in seed parent for traits like stigma exertion, complete panicle exertion etc. which enhances out-crossing is imperative to improve the seed producibility in CMS. Recently, a CMS line IR-79156A possessing more than 50% out-crossing, developed by IRRI showed seed producibility of 3.5 t/ha which needs to be utilized for genetic diversification of CMS to enhance out-crossing.

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

6.2. Research opportunity in HR

6.2.1. Inter-specific hybrids:

Interspecific genetic pool, particularly *indica/japonica* is comparatively more heterotic, however, owingto poor grain quality and HS, this system remain sun exploited. Now it is possible to improve the quality of grain in rice by employing compatible parents having similar grain quality parameters. Besides, through genomics/genome editing interventions, HS problem in inter-subspecific hybrids can be overcome by stacking *indica* allele (*S-i*) at *Sb, Sc, Sd* and *Se* loci and the neutral allele (*S-n*) at *S5*locusin one of the parents (Guoet al., 2016) or by silencing the *S-i* and *S5* mutants through genome editing tools (Shen et al., 2017).

6.2.2. Development of autoplasmic hybrids:

The WA cytoplasm has several heritable abnormalities (CMS load), exerts unwanted effects in hybrids, thus reduces heterosis (5-10%). Iso-cytoplasmic restorer (IR) is fertile segregant of three-line hybrid, harbours same cytoplasm as of CMS utilized in parental hybrid, able to neutralize the fatal cyto-nuclear interaction and enhance the heterosis (Kumar et al., 2017).

6.2.3. Out-crossing improvement in CMS:

The seed parent/CMS existing are poor in out-crossing, restricts seed producibility (mere 1.5-2.5 t/ha) and area expansion of HR. Improving seed parent out-crossing enhancing traits like stigma length and exertion, panicle exertion is workable. Substantial progress has been made in this area, recently, IRRI has developed a CMS, IR-79156A having>50% outcrossing and 3.5 t/ha seed producibility.

6.2.4. Breeding HR with ideal plant type:

This strategy initially started in Japan and China for enhancing genetic gain for yield in rice. This strategy has made substantial progress in the form of 'super rice hybrid' in China as> 100 super hybrids are released (Yuan et al., 2017). Besides, hundreds of genomic regions responding heterosis have been discovered, amongst restores are contributing comparatively more superior alleles than female (Huang et al., 2016). Inclusion of inter-subspecific quality type inbreeds 'super rice' in hybrid development will have substantial impact in attaining quantum genetic gains in hybrids. The QTL, Hd3a and TILLER ANGLE CONTROL1 (TAC1) are found abundantly in restorer lines are key heterosis contributor in *indica* three-line rice hybrids (Krieger et al., 2010; Yu et al., 2007). The locus responding ideal plant architecture1 (IPA1) is found to be ideal HR designing (Zhang et al., 2017). Whereas, allelic combination of genes/QTL, *Ghd8 GW3p6, LAX1, PN2p6/Tms5, OsMADS51* and *SS3p10*are found more heterotic in two *indica* lines; and genes, *Sd1, IPA1 Hd1, TAC1, SS11q20 SS2q25, NAL1* in combination found to be most desirable in *indica/japonica* hybrids (Chen E et al., 2019). It is feasible to modify the key heterosis locus on restorer lines or superior varieties assisted by gene modification (Chen et al., 2019).

7. WAY FORWARD

HR technology has supplemented food security substantially in rice growing countries in temperate zones. However, it could not make substantial dent in tropics where owing to low photon availability during wet season, yielding ability of HR varieties remains meagre. Under changing climatic and agriculture scenario rice hybrid is likely to face stiff competition to sustain in future. Despite having great potential to enhance production and productivity, it has not been adopted on large scale as was expected. This is due to several constraints like lack of acceptability of hybrids across the country due to region specific grain quality requirement. Moderate (15–20%) yield advantage in hybrids is not economically very attractive, needs strategic breeding intervention to increase the extent of heterosis further. Lower market price offered for the hybrid rice produce by millers/ traders is acting as a deterrent for many farmers to take up hybrid rice cultivation. Higher seed cost is another restrain for large scale adoption and hence there is a need to enhance the seed yield in hybrid rice seed production plots. Efforts for

creating awareness and for technology transfer were inadequate in initial stages. Involvement of public sector seed corporations in large scale seed production has been less than expected. Hybrids rice for aerobic/upland, *boro* season and long duration hybrids for shallow lowland conditions to be developed. Most of the constraints mentioned above are being addressed with right earnestness through the on-going research projects and transfer of technology efforts.

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Molecular breeding for diseases and insect pest resistance in rice

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SUMMARY

Rice is major staple food of India, and it needs to be tailored to face the future challenges arising from continuously evolving pests and stresses created by those organisms. The most economically viable and environment friendly management strategy of combating these stresses is exploitation of host plant resistance. The development and availability of an array of robust molecular markers and dense molecular genetic maps in crop plants has made application of marker assisted selection possible for traits governed bymajor genes andQTL. Deployment of these genes/ QTL through resulted in the development of many biotic stress tolerant varieties in rice in India and around the world especially for stresses like bacterial blight, blast, sheath blight, brown plant hopper, gall midge etc. Many multi-gene pyramided lines were developed through MAS which was otherwise impossible through conventional breeding approach. Recent advancements in genome editing technologies have opened up avenues to expand the dimension of molecular breeding.

Keywords: Rice, disease resistance, blast, bacterial blight, sheath blight, gall midge, yellow stem borer, brown plant hopper, molecular breeding, genes and QTL

1. INTRODUCTION

About 23% of the dietary energy requirement of the world human population is met from rice (*Oryza sativa*). Rice is grown in many countries of the world and thus has a major socio-economic impact. To sustain the food security of the ever-increasing population, rice production needs to be enhanced by 30% by the year 2030 (FAO, 2014).Rice productivity is affected by several biotic and abiotic stresses. Diseases and insect pests like blast, bacterial blight (BB), sheath blight (ShB), yellow stem borer (YSB), gall midge (GM) and brown plant hopper (BPH) are the major biotic limitations to rice production, especially in the Indian context. The promotion of monoculture with a concomitant decline in genetic variability and intensive cultivation practices intended for increased rice production has enhanced the vulnerability to biotic stresses. Natural resources are declining, whereas demand for production from per unit land is increasing. Under such constraints, saving

the plants from pests can prove to be a critical component towards meeting the increased demand for food. Thus, aneconomically viable, socially acceptable, and ecologically sustainable approach to combat the problems through the utilization of host plant resistance (HPR) is effective. Breeding for multiple pest-tolerant rice cultivars is one of the importantobjectives of rice improvement programmes worldwide.In modern rice breeding, researchers heavily depend on mapped genes and quantitative trait loci (QTL) to avoid environmental effects on phenotype and high cost for precise phenotyping of many genotypes. Molecular breeding approaches provide the opportunity to combine multiple genes for the same trait in a single background. To date, several genes/ QTL conferring resistance to blast, BLB, sheath blight, BPH and gall midge have been identified, mapped to specific chromosomal location, and tightly linked molecular markers have been reported. Through marker assisted selection (MAS), it is now possible to transfer the resistant genes to elite genetic backgrounds for developing resistant cultivars with better accuracy and speed. In the following sections, we have discussed the available molecular resources to address the important biotic stresses for rice improvement.

2. BLAST DISEASE

Rice blast is the most widespread and devastating disease of rice across the world. The causal organism is *Magnaporthe oryzae*. In 1913, the disease was first reported in India and, in 1919, it caused a major epidemic in the Tanjore delta of erstwhile Madras province in southern India. During the last 100 years, the pathogen and the host plant resistance have been studied in different countries. However, still, it remains as the most destructive disease of rice. Hence, continuous discovery and incorporation of novel resistance genes is essential for mitigating the disease. The gene-for-gene concept (Flor, 1956) for rice *R* gene and *M. oryzae* avirulence (*AVR*) genes were well established. The resistance genes (*R* genes) produce R proteins that interact with the pathogen effector proteins both directly and indirectly. Consequently, host plants recognize the pathogen invasion and induce disease resistance. Some of the examples for the *R* gene-*AVR* gene pair interactions are *Pik*/*AvrPik*, *Pi-CO39*/*Avr1-CO39*, *Pita*/*AvrPi-ta*, *Pia*/*AvrPia*, *Piz-t*/*AvrPiz-t* and *Pi54*/*AvrPi54* (Kanzaki et al., 2012; Cesari et al., 2013; Orbach et al., 2000; Ortiz et al., 2017; Wang et al., 2016, Tang et al., 2017; Ray et al., 2016). The substantial genetic diversity of the causal organism and emergence of new virulent pathotypes causes blast epidemics. Mostly, the chemical fungicides are extensively used for disease management. Although they are effective in controlling diseases, the development of resistance by the pathogen, toxic effect on the environment and human health are the major concern with pesticides. Hence the development of durable resistant cultivars using *R* genes will be the most effective strategy to control rice blast (Wu et al., 2015).

2.1 Sources of blast resistance genes

So far rice researchers have discovered 118 blast resistance(R) genes (Kalia et al., 2019). Approximately, 45% of those genes are identified in *japonica* cultivars and the rest 51% and 4% are from *indica* cultivars including wild *Oryza* species (Kalia et al., 2019). Blast resistance genes (Table 1) of rice have been reviewed by Sharma et al. (2015) and Kalia et al. (2019). The genes are generally found as clusters which are either allelic or tightly linked genes and belong to the nucleotide-binding site–leucine-rich repeat (NBS–LRR) protein family. Liang et al. (2016) reported *pi 66(t)* as the first major gene for resistance. This gene was mapped on chromosome 3 and found to be one of the three recessive genes contributing towards blast resistance. A broad-spectrum race non-specific durable resistance gene contributing to durable high-level resistance to multiple *M. oryzae* races was recently identified (Li et al., 2017) from the genotype "Digu". A total of 80 rice varieties released over last several decades in India were assessed for blast resistance genes diversity and observed 12 major resistance genes (*Pib*, *Piz*, *Piz-t*, *Pik*, *Pik-p*, *Pikm*,*Pik-h*, *Pita/ Pita*-2, *Pi2*, *Pi9*, *Pi1* and *Pi5*) (Yadav et al., 2017). Among those 80 genotypes, 19 were resistant, 21 moderately resistant and the remaining were susceptible. The frequency of presence of resistance genes varied from 0.00-100% among the cultivars. With the inclusion of more diverse genotypes from multiple ecologies (Yadav et al., 2019) using larger number of genes (24 major loci and 28 gene-specific markers), it was found that the frequency of resistant genotypes was lower in this case. Among others, blast resistance genes *Pi56(t)* and *pi21*were strongly associated with resistance. Susan et al. (2018) evaluated major blast resistance genes (18) from 288 land races of north-eastern states. The study grouped the landraces into three categories namely resistant(75 genotypes), moderately resistant (127 genotypes) and susceptible (86 genotypes). The globally discovered genes for blast resistance and their sources have been enlisted in Table 1.

2.2. Introgression of blast resistance genes in commercial cultivars

Although many studies were conducted on blast resistance from landraces, elite cultivars, and wild species, there is still a need to explore more rice germplasm for the improvement of blast resistance. For blast and bacterial blight, Bordeos et al. (1992) transferred resistance genes from the allotetraploid wild rice *Oryza minuta* to *O. sativa*. Marker assisted selection (MAS) is playing a crucial role in getting resistant genotypes that can facilitate gene pyramiding in plants by combining two or more genes. Like many other diseases, marker assisted gene pyramiding is very useful for developing broad-spectrum and durable resistance to rice blast disease. Through gene pyramiding, Hittalmani et al. (2000) introgressed three blast resistance genes *Pi1*, *Piz*-5 and *Pita* into a susceptible cultivar CO39. They found that the plants carrying multiple gene combinations

Table 1. List of blast resistance genes and their genetic location

Sl. No	Gene	Chromosome	Donor	Linked marker	References
1	*Pit*	1	K-59, Tjahaja, K-59	RFLP, SNP	Kaji et al. (1997) and Hayashi et al. (2006)
2	*Pi27(t)*	1	Q14	SSR	Zhu et al. (2004)
3	*Pi-h2(t)*	1	HR4	SSR	Xiao et al. (2015)
4	*Pi-tp(t)*	1	Tetep	SSR	Barman et al. (2004)
5	*Pi35(t)*	1	Hokkai 188	SSR	Nguyen et al. (2006)
6	*Pi64*	1	Yangmaogu	SSR, Indel	Ma et al. (2015)
7	*Pi 37(t)*	1	St. No. 1	SSR	Chen et al. (2005)
8	*Pi-sh*	1	Akihikari	SSR	Fukuta (2004)
9	*Pir2-3(t)*	2	IR64	SSR	Dwinita et al. (2008)
10	*Pirf2-1(t)*	2	*O. rufipogon*	SSR	Dwinita et al. (2008)
11	*Pi-Da(t)*	2	Dacca 6	SSR	Shi et al. (2012)
12	*Pig(t)*	2	Guang-changzhan	SSR	Zhou et al. (2004)
13	*Pi-25(t)*	2	IR64	QTL	Sallaud et al. (2003)
14	*Pi-tq5*	2	Teqing	RFLP	Tabien et al. (2000)
15	*Pi-14(t)*	2	Maowangu	RFLP, Isozyme	Pan et al. (1998) and Zhou et al. (2004)
16	*Pi-16(t)*	2	AUS373	RFLP, Isozyme	Pan et al. (1999) and Zhou et al. (2004)
17	*Pi-d1(t)*	2	Digu	SSR, RFLP	Chen et al. (2004)
18	*Pi-y2(t)*	2	Yanxian No.1	SSR	Lei et al. (2005)
19	*Pi-y1(t)*	2	Yanxian No.1	SSR	Lei et al. (2005)
20	*Pi-b*	2	Tohoku, Koshiihikari	SNP	Hayashi et al. (2006)
21	*Pi66(t)*	3	AS20-1	SSR	Liang et al. (2016)
22	*Pikur 1*	4	Kuroka	Isozyme	Fukuoka et al. (2009)
23	*pi-21*	4	Owariha-tamochi	RFLP, SSR	Fukuoka and Okuno (2001)
24	*Pias(t)*	4	Asominori	SSR, CAPS	Endo et al. (2012)
25	*Pi-45(t)*	4	Moroberekan	SSR	Kim et al. (2011)
26	*Pikahei-1(t)*	4	Kahei	SSR, SNP	Xu et al. (2008a)
27	*Pi-39(t)*	4	Chubu 111	SSR	Terashima et al. (2008)
28	*Pi26(t)*	5	IR64	RFLP, RAPD,	Sallaud et al. (2003)
29	*Pi23*	5	Suweon 365	RFLP, SSR	Ahn et al. (1997)
30	*Pi-10(t)*	5	Tongil	RAPD	Naqvi et al. (1995)

Sl. No	Gene	Chromosome	Donor	Linked marker	References
31	*Pi22*	6	Suweon 365	RFLP	Ahn et al. (1997)
32	*Pi27(t)*	6	IR64	RFLP	Sallaud et al. (2003)
33	*Pi-40(t)*	6	IR65482	STS, SSR	Jeung et al. (2007)
34	*Pi2-1*	6	Tianjingye-shengdao	SSR, SFP	Wang et al. (2012)
35	*Pi2-2*	6	Jefferson	SSR	Jiang et al. (2012)
36	*Pigm(t)*	6	Gumei 4	CAPS, InDel	Deng et al. (2006)
37	*Pi-9(t)*	6	IR31917	STS	Qu et al. (2006)
38	*Pi51(t)*	6	D69	InDel, SSR	Xiao et al. (2012)
39	*Pi2*	6	5173, C101A51	SSR, STS, RFLP	Jiang and Wang (2002) and Zhou et al. (2006)
40	*Piz*	6	Fukinishiki	STS	Zhou et al. (2006)
41	*Piz-t*	6	Toride No. 1	STS	Zhou et al. (2006)
42	*Pi50(t)*	6	EBZ	SSR, CAPS	Zhu et al. (2012)
43	*Pi59(t)*	6	Hoaru	SSR	Koide et al. (2013)
44	*Pi26(t)*	6	Gumei 2	RFLP, SSR	Wu et al. (2005)
45	*Pi8*	6	Kasalath	Isozyme markers, RFLP	Pan et al. (1996a) and Takehisa et al. (2009)
46	*Pi-25(t)*	6	Gumei 2	RFLP, RGA, SSR	Wu et al. (2005)
47	*Pid3*	6	Digu	STS	Shang et al. (2009)
48	*Pi-13*	6	Kasalath	SSR	Ebitani et al. (2011)
49	*Pi-dt(2)*	6	Digu	SSR, RGA	Chen et al. (2004)
50	*Pid2*	6	Digu	CAPS	Chen et al. (2006)
51	*Pi-tq1*	6	Teqing	RFLP	Tabien et al. (2000)
52	*Pi-17(t)*	7	DJ 123	Isozyme marker	Pan et al. (1996b)
53	*Pi-11(t)*	8	Zhai-Ye-Quing	RFLP, RAPD	Causse et al. (1994)
54	*Pi-33*	8	IR64, Bala	SSR, RFLP	Berruyer et al. (2003)
55	*Pi-29(t)*	8	IR64	RFLP, RAPD, Isozyme	Sallaud et al. (2003)
56	*PiGD-1(t)*	8	Sanhuang-zhan 2	SSR, RFLP, RGA	Liu et al. (2004)
57	*Pi-36(t)*	8	Q61	SSR, CRG	Liu et al. (2005)
58	*pi55(t)*	8	Yuejing-simiao 2	SSR, STS	Xiu-Ying et al. (2012)
59	*Pi-5(t)*	9	RIL249	AFLP, RFLP, CAPS	Jeon et al. (2003)
60	*Pi15*	9	GA25	SSR, CRG	Lin et al. (2007b)

Sl. No	Gene	Chromosome	Donor	Linked marker	References
61	*Pi56(t)*	9	SHZ-2	SSR, CRG, SNP	Liu et al. (2013)
62	*Pihk2*	9	Heikezijing	SSR, ILP, InDel	He et al. (2017)
63	*PiGD-2(t)*	10	Sanhuang-zhan 2	SSR, RFLP, RGA	Liu et al. (2004)
64	*Pi28(t)*	10	Azucena	RFLP, RAPD	Sallaud et al. (2003)
65	*Pi-30(t)*	11	IR64	RFLP, RAPD, Isozyme	Sallaud et al. (2003)
66	*Pi-a*	11	Aichi Asahi	SSR, Indel	Zeng et al. (2011)
67	*Pi60(t)*	11	93-11	SSR, InDel	Lei et al. (2013)
68	*Pi-CO39(t)*	11	Co39	SSR, RFLP	Chauhan et al. (2002)
69	*Pi-7(t)*	11	Moroberekan	RFLP	Wang et al. (1994)
70	*Pi-34*	11	Chubu-32	SSR	Zenbayashi et al. (2007)
71	*Pi-38*	11	Tadukan	SSR, AFLP	Gowda et al. (2006)
72	*Pik-h*	11	IRBLkh-K3	SNP	Xu et al. (2008b)
73	*Pi54*	11	Tetep	SSR	Sharma et al. (2005)
74	*Pik-s*	11	Shin 2	SSR	Fjellstrom et al. (2004)
75	*Pi-jnw1*	11	Jiangnanwan	SSR, InDel	Wang et al. (2016b)
76	*Pi-hk1*	11	Heikezijing	SSR	Wu et al. (2013)
77	*Pi43(t)*	11	Zhe733	SSR	Lee et al. (2009a)
78	*Pi-47*	11	Xiangzi 3150	SSR	Huang et al. (2011)
79	*Pik-l*	11	Liziangxin-tuanheigu	SSR, STS, CAPS	Singh et al. (2015)
80	*Pi46(t)*	11	H4	SSR, InDel	Xiao et al. (2011)
81	*Pi-1(t)*	11	Apura, C101LAC	STS, RFLP, SSR, CAPS	Parco (1995), Yu et al. (1996), Fuentes et al. (2008) and Hua et al. (2012)
82	*Pik-m*	11	Tohoku IL4, Tsuyuake	RFLP, SSR	Kaji and Ogawa (1996) and Li et al. (2007)
83	*Pik-e*	11	Xiangzao 143	SSR, InDel	Chen et al. (2015)
84	*Pi-k*	11	Kusabue, Kanto 51	RFLP, InDel, SNP	Hayasaka et al. (1996) and Hayashi et al. (2006)
85	*Pik-p*	11	K60	SSR, CAPS	Wang et al. (2009)
86	*Pi-h1(t)*	11	HR4	SSR, InDel	Xiao et al. (2015)
87	*Pi65(t)*	11	Gangyu 129	SNP, InDel	Zheng et al. (2016)
88	*Pi49*	11	Mowanggu	SSR	Sun et al. (2013)
89	*Pi-44(t)*	11	Moroberekan	RFLP, STS, AFLP	Chen et al. (1999)
90	*Pi18*	11	Suweon365	RFLP	Ahn et al. (2000)
91	*Pi-lm2*	11	Lemont	RFLP	Tabien et al. (2000)

Sl. No	Gene	Chromosome	Donor	Linked marker	References
92	*Pi-6(t)*	12	Apura	RFLP	Yu et al. (1996)
93	*Pi12*	12	Hong Jiao-Zhan	RFLP	Zhuang et al. (1998)
94	*Pi62(t)*	12	Yashiromochi	RAPD, RFLP	Wu et al. (1996)
95	*Pi12*	12	Hong Jiao Zhan	RFLP	Zhuang et al. (1998)
96	*Pi62(t)*	12	Yashiromochi	RAPD, RFLP	Wu et al. (1996)
97	*Pi-tq6*	12	Teqing	RFLP	Tabien et al. (2000)
98	*Pitb*	12	Zixuan	SSR, InDel	Sun et al. (2016)
99	*Pita3(t)*	12	IRBLta2-Re	SSR	Chen et al. (2014)
100	*Pi61(t)*	12	93-11	InDel, SSR	Lei et al. (2013)
101	*Pi58(t)*	12	Haoru	SSR	Koide et al. (2013)
102	*Pita*	12	Yashiromochi	RFLP, RAPD, SNP	Rybka et al. (1997) and Hayashi et al. (2006)
103	*Pita-2*	12	Yashiromochi, Pi No. 4	RFLP, RAPD, SNP	Rybka et al. (1997) and Hayashi et al. (2006)
104	*Pi-24(t)*	12	Zhong 156	RFLP, RAPD, RGA	Zhuang et al. (2002)
105	*Pi-39*	12	Q-15	SSR	Liu et al. (2007b)
106	*Pi-42(t)*	12	DHR9	RAPD, SSR, STS	Kumar et al. (2010)
107	*Pi-19(t)*	12	IRBL19-A	SSR	Koide et al. (2011)
108	*Pi57(t)*	12	IL-E1454	SSR, STS	Dong et al. (2017)
109	*Pi-31(t)*	12	IR64	RFLP, RAPD,	Sallaud et al. (2003)
110	*Pi-48*	12	Xiangzi 3150	SSR	Huang et al. (2011)
111	*Pi51(t)*	12	Tianjingye-shengdao	SSR, SFP	Wang et al. (2012)
112	*Pi67*	12	Tetep	SSR	Joshi et al. (2019)
113	*Pi157*	12	Moroberekan	RFLP	Naqvi and Chattoo (1996)
114	*Pi-20(t)*	12	IR64	SSR	Li et al. (2008)
115	*Pih3(t)*	12	HR4	SSR	Xiao et al. (2015)
116	*PiGD-3(t)*	12	Sanhuang-zhan 2	SSR, RFLP, RGA	Liu et al. (2004)
117	*Pi-41*	12	93-11	SSR, STS	Yang et al. (2009)
118	*Pi-32(t)*	12	IR64	RFLP, RAPD	Sallaud et al. (2003)

including *Piz*-5 showed enhanced resistance to blast compared to the plants which carry only *Piz-5*. Koide et al. (2010) also developed blast resistance line of CO39 by combining two genes i.e. *Pish* and *Pib* which showed better performance. The introgression of *Pi54* gene to the parental lines of rice hybrid Pusa RH 10 (Pusa 6A, Pusa 6B and PRR78), provided resistance to blast in the improved hybrid line (Singh et al., 2011). Divya et al. (2014) introgressed *Pi1*, *Pi2* and *Pi33 genes* in the variety, ADT43. There are several other such examples (Fu et al., 2012; Singh et al., 2013; Tanweer et al., 2015; Xiao et al., 2016).Blast resistance genes *Piz5* and *Pi54* were incorporated successfully into popular basmati rice varieties Pusa Sugandh 5 and PB 1121 which led to further release as Pusa Basmati 1612 and Pusa Basmati 1609 from IARI, New Delhi.

3. BACTERIAL BLIGHT

Bacterial blight (BB) (causal organism *Xanthomonas oryzae* pv. *oryzae* also known as *Xoo*), is a major disease for countries where rice farming is popular. The plants are mostly affected at the active tillering stage resulting in leaf blight or a more severe form of wilting of leaves known as 'Kresek' causing partial or total failure of the crop. Marker assisted breeding for disease resistant varieties have been one of the most efficient and reliable strategies to control BB infection (Khush et al., 1989). Till now 45 BB resistance genes have been identified from various wild and cultivated sources (Pradhan et al., 2020) (Table 2). Many of these resistance genes have been incorporated as a single gene or as a combination into various rice cultivars. The most effective resistance gene *Xa21* against the BB races found in South and Southeast Asia was identified from the wild species *Oryza longistaminata* at ICAR-NRRI (trait identification) and mapped at IRRI, Philippines (Devadath, 1983; Khush et al., 1989). This gene was further cloned and characterized (Song et al., 1995) and has been extensively used worldwide to confer resistance to BB alone or in combination with other genes. Two genes, *Xa33* and *Xa38* were identified from *Oryza nivara* (Natraj Kumaret al., 2012; Bhasinet al., 2012). A new gene was identified from an IR24 mutant "XM14" and designated as *xa42* which was found to be resistant against all Japanese *Xoo* races (Busungu, 2016). Recently many novel BB resistance genes like *Xa43(t)* (Kim et al., 2019), *xa44(t)* (Kim, 2018) and *xa45(t)* (Neelam et al., 2020) were reported. Gene pyramiding through MAS with rapid background recovery without hampering the exquisite quality and characteristics of rice could be a key player for the improvement of disease resistance in the crop. Due to the presence of dominance and epistasis gene action of disease resistance genes, traditional breeding methods make gene pyramiding extremely difficult especially for transferring genes with analogous reactions to two or more races.

3.1. Development and Release of BB resistant Varieties

With closely linked molecular markers, identification of plants with multiple genes for the same trait is possible (Shanti et al., 2010; Singh et al., 2001; Sundaram et al., 2008). The cultivar PR106 has been developed by pyramiding three BB resistance genes (*xa5, xa13* and *Xa21*). Singh et al. (2001) reported that multiple genes pyramided lines showed enhanced resistance against 17 *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) isolates. IRRI developed IR24 NILs (IRBB lines) containing the *Xa4, Xa5, Xa13*, and *Xa21* genes, as well as their combinations, which were widely used in many countries as differential hosts as well as donor source.

At ICAR-NRRI, bacterial blight resistance genes were pyramided into five popular varieties Lalat, Tapaswini, Swarna, IR 64 and Jalmagna. Two varieties namely Improved Lalat [CRMAS 2621-7-1 (IET 21066)] and Improved Tapaswini [CRMAS 2622-7-6 (IET 21070)] were developed through marker assisted selection by pyramiding of four BB resistance genes (*Xa4*, *xa5*, *xa13* and *Xa21*) and released/ notified for Odisha in 2012. They are suitable for growing in the "bacterial blight" endemic areas in the state. CR Dhan 800 was notified in 2019 which carries three genes for BB resistance in the background of mega variety, Swarna (Pradhan et al., 2018).Two resistance genes combinations of *Xa21+xa5* and *Xa21+xa13* showed higher resistance compared to single resistance gene (Pradhan et al., 2016). Three durable and broad spectrum resistance gene combination of *Xa21+ xa13+ xa5* pyramided in the background of Jalmagna and Swarna (Pradhan et al., 2015; Pradhan et al., 2019). Bacterial blight is quite common in basmati rice varieties.A massive breeding programme was undertaken at IARI, New Delhi for incorporating bacterial blight resistance genes into popular basmati varieties like Pusa Basmati 1, Pusa 1121, Pusasugandh 5 and PB 6. Subsequently bacterial blight resistant varieties namelyImproved Pusa Basmati-1, Pusa 1592, PB 1718 and PB 1728 were developed through MAS and released for cultivation.

The mega variety Samba Mahsuri was also improved for bacterial blight resistance and Improved Samba Mahsuri carrying the BB resistance genes *xa5*, *xa13* and *Xa21* was released.The BB resistance genes *xa5*, *xa13* and *Xa21*were also pyramided in 'ADT43' and 'ASD16' (Perumalsamy et al., 2010) and was found effective against two common *Xanthomonas oryzae* isolates from South India. Basavaraj et al. (2010) have used foreground selection markers RG 136 and pTA248 linked to the BB resistance genes *xa13* and *Xa21*, respectively, and improved Pusa 6A by using improved Pusa 6B as a donor for *xa13* and *Xa21*. According to Deshmukhet al. (2017), a high yielding, medium duration rice variety resistant to BB was developed in the genetic background of Karma Mahsuriby transferring three BB resistance genes,*xa5*, *xa13* and *Xa21*. Recently *Xa38* gene was incorporatedinto the genetic background of Improved Samba Mahsuri(Yugander

Table 2. Bacterial blight (BB) resistance genes and linked markers

Sl. No.	Gene	Location (Chr. No.)	Markers	Donor Name	Marker Type	Reference
1	*Xa1*	4	Npb235	IRBB1,Kogyoku and Java14	RFLP	Yoshimura et al. (1998) Sakaguchi S,(1967)
2	*Xa2*	4	HZR950-5HZR970-4	IRBB2	SSR	He et al. (2006)
3	*Xa3/ Xa26*	11	C481S	*Oryza sativa* ssp. *japonica* WaseAikoku 3, IRBB 3/26, Chukogu 45, Minghui 63.	RFLP	Ezuka et al. (1975); Sun et al. (2004)
4	*Xa3/ Xa26 / Xa9/ Xa6*	11	C481SY6855RA	Zhachanglong IRBB3 Minghui 63	RFLP	Sun et al. (2004)Xiang et al. (2006)
5	*Xa4*	11	RM224MP1 MP2Npb181, Npb78	IRBB4	SSRRFLP	Sun et al. (2003), Yoshimura et al. (1995)
6	*xa5*	5	RM13RG556RZ207	DV85, IR1545-248IRBB5	SSRCAPS	Singh et al. (2001)Iyer-pascuzzi and McCouch (2007) Yoshimura et al. (1998)
7	*Xa5(t)*	5		Ajay	SSR	Rao (2003)
8	*Xa7*	6	M1,M5,M3	IRBB7	AFLP, STS,SSR	Porter et al. (2003)
9	*xa8*	7	RM500RM533	IRBB8	SSR	Singh et al. (2002)
10	*Xa10*	11	M491M419	IRBB10Cas 209	RFLPCAPS	Gu et al. (2008)
11	*Xa11*	3	RM347KUX11	IRBB11IR8, RP9-3	SSRCAPS	Goto et al. (2009) Ogawa, T. et al. (1991)
12	*Xa12*	4		Java14		Ogawa et al. (1978)
13	*Xa13*	8	RZ28,RG136Xa13	IRBB13	STS	Zhang et al.

Sl. No.	Gene	Location (Chr. No.)	Markers	Donor Name	Marker Type	Reference
			-prom		Functional marker	(1996) Singh et al. (2012)
14	*Xa14*	4	HZR648-5RM280	IRBB14TN1	SSR	Bao et al. (2010), Taura et al. (1987)
15	*Xa15*			*Oryza sativa* ssp. *japonica* M41 (a mutant line of Harebare), IRBB 15	Mutagenesis	Noda and Ohuchi (1989)
16	*Xa16*			*Oryza sativa* ssp. *indica* Tetep, IRBB 16	Mutagenesis	Noda and Ohuchi (1989)
17	*Xa17*			*Oryza sativa*ssp .*japonica* Asominori, IRBB 17	Mutagenesis	Ogawa et al. (1989)
18	*Xa18*			*Oryza sativa* ssp. *japonica* Toyonishiki, IR24, Miyang 23,IRBB 18	Mutagenesis	Ogawa and Yamamoto (1986)
19	*Xa19*			*Oryza sativa* ssp .*indica* XM5 (induced mutant line of IR24), IRBB 19	Mutagenesis	Taura et al. (1991)
20	*Xa20*			*Oryza sativa* ssp .*indica* XM6 (induced mutant line of IR24), IRBB 20	Mutagenesis	Taura et al. (1991)
21	*Xa21*	11	pTA248 pTA818RG103	IRBB 21, *Oryza longistaminata*	Functional marker	Huang et al. (1997), Ronald et al. (1992)
22	*Xa22(t)*	11	R1506	Zhachanglong	RFLP	Wang et al. (2003) Li et al. (1996)
23	*Xa23*	11	03STS1	*Oryza rufipogon*	STS	Fan et al. (2006)Wang et al.

Sl. No.	Gene	Location (Chr. No.)	Markers	Donor Name	Marker Type	Reference
						(2014a, b)
24	*xa24(t)*	2		DV86, DV85,Aus 295	SSR	Wu et al. (2008) Khush and Angeles, (1999)
25	*Xa25*	12	G1314	Minghui63	RFLP	Chen et al. (2002)
26	*Xa25(t)*	4	RM6748 and RM1153	*Oryza sativa* ssp. *Indica* HX-3, somaclonal mutant), IRBB 25	SSR	Gao et al. (2001, 2005)
27	*Xa26(t)*	11	C481S/Y6855RA	*Oryza sativa* ssp. *indica* NepBha Bong, IRBB 26	RFLP	Lee et al. (2003); Sun et al. (2004)
28	*Xa27(t)*	6	M964 and M1197	*Oryza minuta*	RAPDRFLP	Gu et al. (2004)
29	*xa28(t)*			Lati Sail		Lee et al. (2003)
30	*Xa29(t)*	1	C904 and R596	*Oryza officinalis*	RFLP	Tan et al. (2004)
31	*Xa30(t)*	4	RM317, RM562 RM1341, V88, C189 and 03STS	*Oryza nivara* (IRGC 81825) *Oryza rufipogon*	SSRSSR, EST and STS	Cheema et al. (2008) Jin et al. (2007)
32	*Xa31*	4	G235, C600	Zhachanglong	RFLP	Wang et al. (2009)
33	*Xa32*	11	RM6293, RM5926	*Oryza australiensis* introgression line C4064	SSR	Zheng et al. (2009)
34	*Xa33*	7	RMWR7.1 and RMWR7.6.	*Oryza nivara*	SSR	Natraj Kumar et al. (2012)
35	*Xa34(t)*	1	RM10929 and BGID25	BG1222	SSR	Chen et al. (2011)
36	*Xa35(t)*	11	RM144 RM6293 RM7654	*Oryza minuta* (Acc. No. 101133)	SSR	Guo et al. (2010)
37	*Xa36*	11	RM2136, RM7443, RM1233, and RM224	C4059	SSR	Miao et al. (2010)

Sl. No.	Gene	Location (Chr. No.)	Markers	Donor Name	Marker Type	Reference
38	*Xa38*	4	RM17499 Os04g53060	*Oryza nivara* acc. IRGC 81825	SSR Gene based marker	Bhasin et al. (2012)
39	*Xa39*	11	RM26985 and DM13	FF329	SSR	Zhang et al. (2015)
40	*Xa40*	11	RM27320 and ID55.WA18-5	IR65482-7-216-1-2	SSR	Kim et al. (2015)
41	*xa41(t)*	11		*Oryza barthii, Oryza glaberrima*		Hutin et al. (2015)
42	*xa42*	3		XM14		Busungu et al. (2016)
43	*Xa43(t)*	11	IBb27os11_14 and S_BB11.ssr_9	P8 of JMAGIC population	SSR	Kim et al. (2019)
44	*xa44(t)*	11	11964077 and 11985463		SNPs	Suk-Man Kim, (2018)
45	*xa45(t)*	8	LOC_Os08g 42410C8.26737175 and C8.26818765. The peak marker, C8.26810477	*Oryza glaberrima* accession IRGC 102600B	STS, SNPs	Neelam et al. (2020)

et al., 2018).The *Xa33* gene was pyramided with *Xa21* gene to confer a high resistance against BB in the elite maintainer line, DR17B (Balachiranjeevi et al., 2018). Recently rice variety PR 127 has been developed by introgressing*xa 45 (t)* gene into popular variety Pusa 44 (Neelam et al., 2020).

4. SHEATH BLIGHT

Sheath blight (ShB) is a major diseasein areas following intensive rice cultivation across temperate, subtropical, and tropical climates with stagnant water. ShB disease is considered the next most significant rice disease after blast andreduces yield and grain quality worldwide (Molla et al., 2013). Sheath blight tends to be more destructive in fields planted with high-yielding, high-tillering, and nitrogen responsive varieties. A high rate of nitrogen fertilizer promotes luxuriant crop growth with dense canopies. This type of canopy structure has high relative humidity which provides a favourable microclimate for rice sheath blight to occur. High seeding rates and close plant spacing favour the spread of disease because, aside from creating a favourable microclimate, it allows more tissue contacts and longer durations of leaf wetness.

4.1. Sources of resistance

Despite a large number of germplasm screened, there is still no reliable source of complete resistance. More than 30,000 lines and varieties were screened at IRRI for ShBresistance,but no single accession is found to be fully resistant to disease rather many showed moderate resistance. Prasad and Eizenga (2009) evaluated 73 wild *Oryza* spp. accessions and identified three accessions of *O.Nivara* and one each of *O. barthii*, *O. meridionalis* and *O. officinalis* showing moderate resistance. Ram et al. (2008) screened 22 *Oryza* accessions from 11 different species and found that *O. latifolia, O. grandiglumis, O. nivara,* and *O. rufipogon* accessions had the highest degree of resistance. Moderate ShB resistance were found only in few rice cultivars. Pan et al. (1999) identified that TeQing and Jasmine85, two resistant cultivars, both had a non-allelic dominant main resistance gene which segregated independently. Recently, a novel resistant source, CR 1014 for ShB resistant has been reported by Bal et al. (2020) from ICAR-NRRI, Cuttack.

4.2. Identification of QTL

Li et al. (1995) first reportedthe identification of ShB-QTL using RFLP markers and field evaluations derived from a cross between 'Lemont' and 'TeQing'. Previous studies suggested that many ShB resistance QTL were identified using segregating F_2 populations, double haploid (DH), and recombinant inbred line (RILs), back cross populations over all the rice chromosomes. This has been reviewed by Srinivasachary et al. (2010).

Nearly 200 sheath blight resistance QTL have been reported from different countrieswhich showed limited contribution to phenotype (Zarbafi and Ham, 2019; Mollaet al., 2020). Li et al.(1995) reported six QTL from 'TeQing' which collectively contribute for approximately 47% towards PVE in the F_4populations of Lemont/TeQing. Pinson et al. (2005) reported 12 QTL from the same cross, each of those determining 5–18% of PVE.Pan et al. (1999) identified three major QTL (Rh-2, Rh-3, and Rh-7) from Lemont/Jasmine 85 population and the QTL were physically located on chromosomes 2, 3, and 7 contributing to the tune of 14.4%, 26.1%, and 22.2% of the phenotypic variation, respectively. From the same cross, Zou et al. (2000) mapped six QTL andfound that two out of those six QTL are contributed by susceptible genotype Lemont. The other genotypes which were utilized for mapping QTL are Jingxi-17 (Kunihiro et al., 2002), Minghui 63 (Han et al., 2002), 4011 (Che et al., 2003), WSS2 (Sato et al., 2004), Pecos (Sharma et al., 2009), Tetep (Channamallikarjuna et al., 2010), HJX74 (Zhu et al., 2014), RP2068-18-3-5 (Nymagoud 2015), ARC10531 (Yadav et al., 2015) etc.

When it is compared with other major diseases of rice, the number of identified genes/QTL appears negligible (Mollaet al., 2019). However, identification of resistant sources and its transfer to elite rice variety through MAS has become an important strategy to combat this deadly disease. It is now comparatively easier to map genes/QTL due to advancements in phenotyping and genotyping techniques and is sometimes considered as routine. However, mapping genes/QTL for sheath blight from novel sources is still very important Bal et al. (2020) recently reported a stable QTL *qShB-1.1* with 26.9 % PVE for relative lesion height (RLH) under artificial inoculation from a new genotype CR 1014.

4.3. Breeding Approach

The molecular markers linked with ShB-QTL first needs validation in independent breeding populations and diverse germplasm panels before their use in breeding programmes. Reports of such validations are rare for this disease. The effect of the QTL *qSBR11-1*from Tetep was validated in an alternativesusceptible genotype background (F_2 progeny of Pusa-Basmati 1 / Tetep) and a panel of 96 rice cultivars with different degrees of sheath blight resistance (Channamallikarjuna et al., 2010).The QTL*qSB-9TQ*, from an *indica* cultivar TeQing was introgressed byPinson et al. (2008) into three susceptible cultivars and have been released for cultivation in the USA. The genotypes TIL:455, TIL:514 and TIL:642 generated from a biparental cross of 'Lemont' (PI 475833) and TeQingcarry eight novel QTL alleles for sheath blight resistance.Combining well-characterized sheath blight resistance QTL in high-yielding backgrounds through marker assisted selection may help in developing sheath blight resistant cultivars. Alternatively, transgenic offers a scope for the development of sheath blight resistant cultivars. Pathogenesis-related (PR)

Table 3. List of QTL identified for sheath blight tolerance in rice

Sl. No.	QTL Name	Location (Chr. No.)	Markers	Marker Type	References
1	*qShB-1.1*	1	RM11935-RM11968	SSR (120)	Bal et al. (2020)
2	*qShB-1.2*	1	RM11069-RM11312		
3	*qShB-1.3*	1	RM10037-RM10047		
4	*qshb1.1*	1	RM151- RM12253	SSR (70)	Yadav et al. (2015)
5	*qshb7.1*	7	RM81-RM615		
6	*qshb7.2*	7	RM10-RM2169		
7	*qshb8.1*	8	RM21792- RM310		
8	*qSBL7(E2)*	7	D760-RM248	SSR (52) and	Wen et al. (2015)
9	*qSBPL-7 (E2)*	7	D760-RM248	InDel (128)	
10	*qSBD-12-2 (E1)*	12	RM1246-D1252		
11	*qHZaLH3*	3	RM143-RM514	SSR (214)	Zeng et al. (2015)
12	*qHZaDR8*	8	RM1376- RM4085		
13	*qHZaDR9*	9	RM444-AGPSMA		
14	*qHZbDR5*	5	RM3321- RM3616		
15	*qSB-9TQ*	9	CY-85 and Y86	InDel and CAPS (22)	Zuo et al. (2014a)
16	*qDR-4*	4	RM1155- RM5757	SSR (163)	Liu et al. (2014)
17	*qRLL-4*	4	RM1155- RM5757		
18	*qRLH-4*	4	RM1155- RM5757		
19	*qSB-11LE*	11	Z22-27C and Z23-33C	STS and) CAPS (26	Zuo et al. (2013)
20	*qRTL3*	3	RM570	SSR (151)	Taguchi-Shiobara et al. (2013)
21	*qRTL5*	5	RM5784		
22	*qRTL6*	6	RM1161		
23	*qRTL9*	9	RM6251		
24	*qRTL3*	3	RM16200		
25	*qRTL6*	6	RM2615		
26	*qRTL12*	12	RM7025		
27	*qRTL5*	5	RM3286		
28	*qRTL6*	6	RM6395	SSR (199)	Liu et al. (2013)
29	*qRTL9*	9	RM3533		
30	*qShB2-1-AR*	2	RM279–RM71		
31	*qShB7-AR*	7	RM5711–RM2		
32	*qShB7-LA*	7	RM5711–RM2		

Sl. No.	QTL Name	Location (Chr. No.)	Markers	Marker Type	References
33	*qShB11-1-TX*	11	RM7203–RM536		
34	*qShB11-2-TX*	11	RM536–RM229		
35	*qShB6 (wild 1 - field 2009)*	6	RM3431-RM3183	SSR (131)	Eizenga et al. (2013)
36	*qShB6 (wild 2-field 2009)*	6	RM253–RM3431		
37	*qShB6 (wild2-field 2008)*	6	RM253–RM3431		
38	*qShB1 (wild 2-field 2008)*	1	RM431–RM1361		
39	*qShB6-mc (wild1-microchamber)*	6	RM3183– RM541		
40	*qsbr_2.1*	2	RM8254-RM82521	SSR (111)	Nelson et al. (2012)
41	*qsbr_2.2*	2	RM3857- RM5404		
42	*qsbr_12.1*	12	RM3747- RM27608		
43	*qSBR1*	1	RM11229 and Indel (1)	SSR (154)	Jia et al. (2012)
44	*qSBR11*	11	RM7203		
45	*qSBR1-1*	1	RM5389- RM3825	SSR (123	Fu et al. (2011)
46	*qSBR2-1*	2	RM5340-RM521		
47	*qSBR2-2*	2	RM110-osr14		
48	*qSBR2-3*	2	RM7245- RM5303		
49	*qSBR4*	4	RM3288- RM7187		
50	*qSBR5-2*	5	RM7446- RM3620		
51	*qSBR7*	7	RM1132-RM473		
52	*qSBR8*	8	RM8264- RM1109		
53	*qSBR9*	9	RM23869- RM3769		
54	*qShB1 (2007/ 2008)*	1	RM431- RM12017	SSR (227)	Xu et al. (2011)
55	*qShB2 (2008)*	2	RM174-RM145		
56	*qShB3 (2007)*	3	RM135-RM186		
57	*qShB5 (2007)*	5	RM18872- RM421		

proteins were overexpressed through transgenic approach and showed significant improvements. The genes include chitinase (PR-3), b-1,3-glucanases (PR-2), thaumatin- like proteins (PR-5) etc. which have been used for genetic transformation of IR72, IR64, ChinsurahBoro II, Basmati 122, Swarna and IR58 and showed improved ShB resistance. Although encouraging results were recorded during *in vitro* assays, open or contained field trials have not been conducted in those cases and commercial transgenic sheath blight resistant genotypes have not been released anywhere in the world (Srinivasachary et al., 2010).

5. GALL MIDGE

Asian rice gall midge (*Orseoliaoryzae*) is a sucking pest of rice which causes severe yield loss to the crop. In India, damage to crop by this pest is recorded from wide geographical areasand considered as a major pest at most locations. For better management of this pest, the resistance breeding approach is quite useful and ecologically acceptable. Several resistant rice varieties have been developed and released in India (Bentur et al., 2003). Extensiveuse of single resistance gene introgression inmany genotypescauses evolution of new biotypes. These newly emergentbiotypes of the pest are more destructive in natureand overcome the advantages of the deployment. That is why new breeding strategies were needed to overcome this problem and to get durable gall midge resistance. Extensive research has been undertaken to identify GM resistance sources which have resulted in 11 rice GM resistance genes *{Gm1, Gm2, gm3, Gm4, Gm5, Gm6, Gm7, Gm8, Gm9, Gm10 and Gm11(t)}* and fine mapping of some genes resistance genes (Table-4).

The GM resistance gene*Gm2*was identified and mappedto a 0.66-Mb region of chromosome 4 in 'Phalguna' rice within flanking markers RM17473 and RM17503 (Mohan et al., 1994; Yasala et al., 2012). *Gm3,* having markers interval RM17480 and *gm3SSR4* of chromosome 4 was identified in rice line 'RP2068-18-3-5' (Sama et al., 2014). A candidate gene *Os04g52970* for *gm3* was also identified by Sama et al., 2014.Himabindu (2009) tagged the gene *Gm4* from rice genotype 'Abhaya' on chromosome 8 in the marker interval of RM22551 and RM22562 and reported *Os08g09670* as the putative candidate gene. Li et al. (2019) identified *Gm6* from the genotype 'Kangwenqingzhan' and mapped itto chromosome 4 with flanking markers YW91 and YW3-4. Another GM resistance gene *Gm8* was reported from the landrace 'Jhitpiti' located between the microsatellite markers RM22685 and RM22709 of chromosome 8 (Sama et al., 2012), was identified in and mapped to the short arm of chromosome 8 between the markers RM22685 and RM22709. Divya et al. (2018) studied the association between *Gm8* and the proline-rich protein gene in the resistant genotype Agnani and developed a functional marker "PRP-del"for use in MAS. *Gm11(t)* of chromosome 12 was

identified in the rice line 'CR57-MR1523' and mapped in the marker interval of RM28574 and RM28706 (Himabindu et al., 2010).The remaining genes (*Gm5, Gm7, Gm9, and Gm10*) are yet to be mapped. More number of genes from different sources needs to be identified and mapped for developing comprehensive resources (Zhou et al., 2020).

After evaluation of more than 25000 accessions of rice germplasm from National gene bank, nearly 500 resistant genotypes were identified (Bentur et al., 2011; Bentur et al., 2015) with a higher frequency of resistant lines from North east India. Traditional rice breeding approaches for GM resistance were followed in late fifties, leading the release of first GM resistant variety, "Kakatiya," in 1975. Germplasm lines were screened at NRRI, Cuttack and identified donors lines namely PTB2, PTB 10, PTB 18, PTB 21, PTB 24, EswarKorra, Leaung-152, W 1263, Peykeo E63, Peykeo P129 and Siam 29. Shakti and Samalei GM resistant varieties were developed using these resistant donors *viz.*, Other varieties released by the Institute showing resistance to GM were Tara, Neela, Shaktiman, Moti, Kshira, Kalashree, Heera, Gayatri, Dharitri, Udaya and Sarasa.

Nair et al. (2011) established the applicability of gene-for-gene hypothesis in case of resistance genes in rice and different biotypes of the pest. It was clearly established that there are no R genes which is resistant against all biotypes. Similarly, no biotype was found to be virulent against all the reported R genes.Based on the similarity in level of resistance, R genes were categorized into four groups. Among the 11 reported R genes for gall midge, only two genes (*Gm1* and *Gm8*)are HR- type. Rest of the genes belong to HR+ category. Hence significant diversity exists for both ranges and types of R genes for gall midge.

The resistance genes like*Gm4* and *Gm8* are present on chromosome 8 and four genes (*Gm2, gm3, Gm6, Gm7*) exist as a cluster on chromosome 4. Most of these genes have flanking markers with which it will be easier to transfer the genesthrough MAS to different elite rice cultivars. For three genes, the candidate genes have also been identified [*viz., gm3* as NB-ARC (LOC_*Os04g52970.1*), *Gm4*as NBS-LRR (LOC_*Os08g09670.1*) and *Gm8*as proline rich protein (LOC_*Os08g15080*)]and further cloned and characterized(Sama et al., 2014; Divya et al., 2015; Divya et al., 2018).The functional markers for these genes have been designed depending on the gene sequences and will be useful for further breeding programmes (Divya et al., 2015). Different combinations of these genes may be pyramided in a common background to study their effectiveness towards increasing the resistance level.

6. BROWN PLANT HOPPER (BPH)

Brown plant hopper (BPH) (*Nilaparvata lugens* Stål) causes severe damage to rice crop and occasionally destroy the entire crop in the BPH endemic regions.The

Table 4. List of genes conferring resistance to gall midge of rice

Gene	Chromo-some	Markers	Donor	Marker type	References
Gm1	9	RM23941, RM23956	W1263	SSR	Yasalaet al. (2012)
	9	RM219, RM316 & RM444	Kavya	SSR	Birdaret al. (2004)
Gm2	4	Rm17473 & RM 17503	Phalguna	SSR	Mohan et al. (1994)
Gm3	4	RM17473, RM17480 & RMgm3SSR4	RP-2068-18-3-5	SSR	Sama et al. (2014)
Gm4	8	RM22551 & RM22562	Abhaya	SSR	Himabindu (2009)
Gm6	4	YW91 & YW3-4	Kangwen-qingzhan		Li et al. (2019)
Gm8	8	RM22685 & RM22709	Jhitpiti		Sama et al. (2012)
Gm11(t)	12	RM28574 & RM28706	CR57-MR1523′		Himabinduet al. (2010)

mildly infested plants show the symptoms of leaf yellowing, reduction of plant height, growth, vigour and number of active tillers. During heavy infestations complete drying and mortality of plants (hopperburn) is recorded (Watanabe and Kitagawa 2000; Ali et al., 2012). Besides causing direct damage as a pest, the insect also acts as a vector for viral diseases liketungro, grassy stunt, and ragged stunt of rice (Khush and Brar 1991; Rivera et al., 1996; Normile 2008).BPH infestations have become more widespread in Asia in recent years, resulting in significant yield penalty. Due to the vulnerability of common rice varieties to planthoppers, farmers rely solely on chemical pesticides to control this insect, which are costlyand has environmental impact. Furthermore, overuse of pesticides eliminates natural predators which control the insects, resulting in pest revival. Growing resistant rice varieties is the most cost-effective and environmentally safe way to handle this pest.

6.1. Source of genes for BPH resistance

The genetic basis of resistance has been studied widely, and nearly 38 major resistance genes and, many quantitative trait loci (QTL) have been recorded in the gene pool of cultivated and wild rice species(Table-5).

Table 5. List of rice varieties including cultivars, introgressed lines and wild species carrying identified BPH resistance genes.

Sl. No.	Resistance gene	Location	Variety	Markers	References
1	*Bph1*	12	Mudgo, CO22 (IT 000588), TKM6, Milyang30, Milyang34 (IT006216), Nampungbyeo, Chilseongbyeo, Andabyeo, Kanto PL4 (IT173362), Cheongcheongbyeo, Changsongbyeo, Baekunchalbyeo, IR26 (IT001886), IR28 (IT001892), IR29 (IT001893), IR30 (IT001899), Hangangchalbyeo, Yeongpungbyeo, Namyeongbyeo, Gayabyeo, Samgangbyeo, Namcheonbyeo, MTU15, IR26, IR28, IR29, IR30, IR34, IR44, IR45, IR46, IR64 and MGL2	RM247, RM28008, RM7102	Cha et al. (2008)
2	*bph2*	12	ASD7, ASD9, IR 1154-243, Norin-PL4, Hwacheongbyeo, PTB18, PTB33, H105, Palasithari 601, H5, IR32, IR36, IR38, IR40, IR42, IR48, IR50, IR52, IR54, IR65	RM463, RM3331	Jena et al. (2017)
3	*Bph3*	6	Rathu Heenati, PTB19, Gangala, HoranaMawee, Muthumanikam, Kuruhondarawala, Mudu, Kiriyal, PTB33, IR56, IR58, IR60, IR62, IR68, IR70, IR72, IR74	RM586, RM589	Xiao et al. (2016)
4	*bph4*	6	Babawee, Gambada Samba, Hotel Samba, Kahata Samba, Thirissa, Sulai, VellaiIllankali, Heenhoranamawee, KuluKuruwee, Lekam Samba, Senawee and IR66	RM589, RM586, RM190	Jena et al. (2017)
5	*bph5*	6	ARC10550	RM16999 RM5757 RM17008	Deen et al. (2017)
6	*Bph6*	4	Swarnalata, *O. officinalis* (acc.00896)		Guo et al. 2018
7	*Bph7*	12	T12	RM28295 & RM313	Qiu et al. (2014)
8	*bph8*		Chin Saba, Col. 5 Thailand and Col. 11 Thailand		Nemoto et al. (1989)
9	*Bph9*	12	Pokkali, Balamee and Kaharamana	RM5341, RM463, RM28502, InD2	Jena et al. (2017)

Sl. No.	Resistance gene	Location	Variety	Markers	References
10	*Bph10*	*12*	*O. australiensis* and IR65482-4-136-2-2	RG457, RM277, RM260	Jena et al. (2017)
11	*bph11*	*3*	*O. officinalis*, DV85 and IR 54751-2-44-15-24-3		
12	*Bph12*	*4*	*O. officinalis, O. latifolia,* B14 and IR54751-2-34-10-6-2	RM1305	Hu et al. (2016)
13	*Bph13*	*3*	*O. eichingeri, O. officinalis* (acc.00896, acc. 105159) and IR54745-2-21-12-17-6	RM517	Hu et al. (2016)
14	*Bph14*	*3*	*O. officinalis*, RI35 and B5	*IN76-2*	Du et al. (2009)
15	*Bph15*	*4*	*O. officinalis* and B5	IN15-6	Hu et al. (2012)
16	*Bph17*	4	Rathu Heenati	RM518, RM8213, RM5953, RM401XC4-9, IN15-6	Jena et al. (2017)Xiao et al. (2016)
17	*Bph18*	*12*	*O. australiensis* and IR65482-7-216-1-2	7312.T4A RM3331, RM28427	Ji et al. (2016)
18	*bph19*	3	AS20-1	7312.T4A, BPH18-ind2	Jena et al. (2017)
19	*Bph20*	*4*	*O. minuta* (acc. 101141), IR71033-121-15 and ADR 52	S4019XY 4-17, HJ28	Jena et al. (2017) Xiao et al. (2016)
20	*bph21*	12	ADR52, *O. minuta* (acc. 101141) and IR71033-121-15	S12094 HJ12/HJ9	Jena et al. (2017) Xiao et al. (2016)
21	*Bph22*		IR 75870-5-8-5-B-2-B and IR 75870-5-8-5-B-1-B		
22	*Bph23*	6	IR 71033-121-15		
23	*bph24*	4	IR 73678-6-9-B	HJ22 and RM16717	Xiao et al. (2016)
24	*Bph25(t)*	6	ADR52	J22, 16717	Xiao et al. (2016)
25	*Bph26(t)*	12	ADR52	RM309, RM28449, S20103, RM5479, MSSR2	Jena et al. (2017)
26	*Bph27*	4	GX2183		Huang et al. (2013)
27	*Bph28(t)*	4	DV85	RM16853	Wang et al., (2015)

Sl. No.	Resistance gene	Location	Variety	Markers	References
28	*Bph29*	10	RBPH54 (introgression from *O.rufipogon*)	RM244	Wang et al. (2015)
29	*Bph30*	4	AC-A613	RM16278 and RM16425	Wang et al. (2018)
30	*Bph31*	3	CR2711-76	Indel PA26 and RM2334	Prahalada et al. (2017)
31	*Bph32*	6	PTB33	RM589, RM588, RM8072, PASH6	Jena et al. (2017)
32	*Bph33*	4	IRGC 36295, IRGC 36352	RM551, RM16994 RM17006	Naik et al. (2018)
33	*Bph34*	4	*Oryza nivara* (acc. IRGC104646)	RM16994, RM17007	Kumar et al. (2018)
34	*Bph35*	4	*Oryza rufipogon*, RBPH660		Yuexiong et al. (2019)
35	*Bph36*	4	Wild rice GX2183		Li et al. (2019)
36	*Bph37*	1	IR64		Yang et al. (2019)
37	*Bph38(t)*	1	Khazar		Balachiranjivi et al. (2019)

6.2. BPH resistance genes / QTL

Since the 1970s, large no. of rice germplasm including wild rice species was screened against BPH at IRRI(Jackson, 1997). The mechanism of host-plant resistance between *N. lugens* and rice has been widely explored since its first discovery in 1960 (Pathak et al., 1969). Recently three resistance genes *viz*., *Bph33* (Hu et al., 2018), *Bph36* (Li et al., 2019) and *Bph38(t)* (Balachiranjeevi et al., 2019) have been identified. The resistance genes identified till date are distributed on chromosome # 2, 3, 4, 6, 11 and 12 of rice (Cheng et al., 2013). A total of 20 resistance genes have been fine-mapped (*BPH14, BPH17, BPH18, BPH26, BPH29, BPH9, BPH32* and *BPH6*) and eight have been cloned and characterized (Balachiranjeeviet al., 2019). Resistance QTL/genes from two landraces of Odisha identified as*qBph4.3* and *qBph4.4* were from Salkathi (Mohanty et al., 2017) and *BPH31* from *Dhobanumberi* (Prahlada et al., 2017).

6.3. Strategies to develop BPH resistance varieties through molecular breeding

As the new BPH biotypes easily break down the monogenic resistance, BPH management is a serious concern for better rice productivity in Asian countries. Rice varieties with long lasting resistance should be grown in the particular areas for overcoming this threat. Gene pyramiding through MAS utilizing effective BPH resistance genes will help in developing varieties with durable resistance. Previous studies suggested that many improved cultivars (e.g. IR26, IR36, IR50 and IR72) were released carrying BPH resistance genes such as *Bph1, bph2, Bph3* and *bph4* at IRRI (Jeunget al., 2007; Jena and Kim, 2010). Marker-assisted breeding was used for pyramiding of BPH resistance genes *Bph1* and *Bph2* (Sharma et al., 2004). It was recorded that, the NILswith two pyramided resistance genes, *Bph14* and *Bph15,* showed a higher degree of resistance than the lines containing single gene (Li et al. 2006; Braret al., 2009). Myint et al. (2012) also reported multi gene pyramiding of the BPH resistance genes *BPH25* and *BPH26* showing higher level of resistance. The intercrossing of single-gene introgressed lines carrying *Bph27(t)* and a major BPH resistance gene *Bph3* with marker aided pyramiding greatly increased BPH resistance and decreased yield loss caused by the insect (Liu et al., 2016). Fan et al. (2017) pyramided BPH resistance genes *Bph6* and *Bph9*, along QTL for yield and fertility restorer genes through MAS and developed broad spectrum BPH resistant restorer lines of hybrid rice. Jena et al. (2017) developed 25 NILs of BPH susceptible *indica* rice cultivar IR24 with multiple resistance genes against BPH. Nine genotypes were carrying single R genes and 16 were carrying multiple R gene combinations (11 two-gene pyramids and 5 three-gene pyramids).

ICAR-NRRI, Cuttack is also working on developing BPH tolerant varieties from Indian landraces. The breeding lines CR 3005-77-2 (Samba Mahsuri / Salkathi), CR 3006-8-2 (Pusa 44/Salkathi), CR 3005-230-5 (Samba Mahsuri/ Salkathi), CR 2711-76 (Tapaswini/ Dhobanumberi) showed encouraging results in the AICRIP BPH screening trials. Transfer of QTL from Salkathi (*qBph4.3* and *qBph4.4*) into the backgrounds of popular varieties Naveen and Pooja through MAS is on going.

7. YELLOW STEM BORER (YSB)

Stem borers (Lepidoptera) are ubiquitous in most rice-growing regions and inflicts significant yield loss. The most commonstem borer species found in India are yellow stem borer (*Scirpophaga (Tryporyza) incertulas*), striped stem borer (*Chilosuppressalis*), and pink stem borer *Sesamia inferens* (Listinger 1979). *S. inferens* is found mainly in northern India's hill regions and eastern India's Bengal belt (Banerjee and Pramanik 1967). Rarely, other species like the white rice borer (*S. innotata*) are also found in hilly areas. The yellow stem borer (YSB), *S. incertulas* is

the predominant species in India and is the major pest of waterlogged ecologies.

Varietal resistance to YSB has been investigated by many workers from the last six decades. In India, mostly three rice landraces namelyi.e., TKM 6, CB1 and CB2 have been used as resistant donors since 1964 (Sampath et al., 1970). The mechanism of resistance in TKM 6 was reported to be partially for non-preference and antibiosis (DRR, 1969). Due to the non-availability of complete resistance for YSB in rice, it is challenging for the breeders to develop resistant varieties. Also, the instinctive screening challenges and diverse genetics behaviour of YSB make it more difficult for resistance breeding.Transgenic plants expressing genes for *Bacillus thuringiensis* (Bt) -endotoxins, protease inhibitors, enzymes, and plant lectins have shown insect resistance. In China, insect resistant GM rice has shown to be highly effective in trials. Sarwar (2012) found that aromatic rice varieties were more susceptible to YSB than non-aromatic varieties. The increased feeding activities of the larvae, especially the fifth instar larvae, which are the most voracious feeders, caused high stem borer damage to aromatic varieties. Antibiosis (reduced lifespan and fertility, increased insect mortality), antixenosis (reduced plant attractiveness), and resilience (ability to tolerate a pest) may all contribute to stem borer resistance in non-aromatic varieties (Padmakumari et al., 2017).

7.1. Introgression of YSB resistance genes from wild species

YSB resistance is almost absent in the primary gene pool of rice. While some high-yielding rice varieties are reported as moderately resistant, complete YSB-resistant rice variety is not reported in cultivated rice.Therefore, search for tolerance source from wild rice is a priority area of research. Some earlier studies suggested that, *O. brachyantha, O. officianalis, O. ridleyi* and *Oryza coarctata* are resistant against YSB (Brar et al., 1996; Mondal et al., 2018 and Padhiet al., 2002). Attempts to introgress YSB resistance from *O. ridleyi* were unsuccessful (Brar et al., 1991). Narain et al. (2015) used an African wild species, *Oryza brachyantha* (FF) to develop monosomic alien addition lines (MAALs) with target for transferring YSB resistance trait to cultivated rice. However, the non-preference of *O. Brachyantha*is mainly due to its slender culms which are not suitable for larvae of the insect to grow inside.

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Genetic solutions to improve nutrient use efficiency in rice

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SUMMARY:

Crop improvement takes advantage of heritable variations and the genetics that governs them. India as a country has achieved its self-sufficiency in cereal production, particularly in rice, despite overlooking many critical aspects. Nutrient use efficiency is one such vista that is yet to be explored. This chapter aims at highlighting the role of essential nutrients such as nitrogen, phosphorus, iron, and zinc required for rice growth and the genes/transporters involved in facilitating them. For instance, NRT and AMT transporter families for nitrogen uptake, PHT transporters for phosphorus uptake from the shallow layers of soil, etc. Such a crop improvement scheme would require activities like germplasm evaluation, identifying the genetics behind the traits of interest, the genomic regions harboring them, and finally a suitable donor parent to transfer the gene into a desirable background genome. There have previously been examples of such efforts undertaken in the scientific community (discovering *PSTOL1* gene, evaluation of germplasm for *TOND1* gene etc.) but still, a lot needs to be done in this direction.

Keywords: Heritable, Nutrient use efficiency, germplasm, phosphorus uptake, PSTOL1, TOND1

1. INTRODUCTION

The burgeoning population and the increasing demand for food compelled crop growers to adopt intensive agriculture. Therefore, they relied much on the exploitation of resources like soil, water and chemical supplements. This goes against the policies for sustainable development with improving soil health and water quality. Hence, it is difficult to ignore the increasing reliance on chemical supplements for sustainable growth and development in rice. The reducing genetic potential of the varieties, which were developed to utilize nutrients efficiently and now to survive under nutrient-deficient soils. The breeding activities so far have been focused to develop high-yielding genotypes and the genetic potentialities of these varieties are expressed by ample supply of inputs externally (fertilizers, pesticides, and irrigation water). This practice has made them more dependent on external applications rather than adapting to limited input conditions. But, the existing environmental dynamics and crisis of climate change suggest

otherwise. In the coming decades, the focus should be on developing and/or improving genotypes that can survive under limited external inputs by utilizing the available resources to the optimum, maintaining a balance between profitability margins and essential resource input costs.

Rice (*Oryza sativa* L.), the second most cultivated crop nurtures billions of lives including human and animal and occupies prime importance in developing nations especially in Asia where it is the sole source of energy (carbohydrate) for the majority of the population. The nutritional content of rice grain is 80% carbohydrates, 7–8% protein, 3% fat, and 3% fiber (Chaudhari et al., 2018). The modern high-yielding rice genotypes are adapted to high doses of fertilizers and as seen under the field conditions that the nutrient use efficiency is less than 50% (of the total applied fertilizers). For instance, nitrogen recovery efficiency remains below 30% under rain-fed conditions, whereas it ranges between 20-30% under irrigated ecology (Roberts, 2008). Similarly, for phosphorus (P), the observed trend over the past few decades indicates that the P use efficiency has gone down to as low as 10-20% (Li et al., 2009) and hence the reliance on P fertilizers has increased. This is either due to the loss of the nutrients through drainage, volatilization, leaching, etc., or fixation of P in the soil making them unavailable to the plants. Under this situation, the option remains either to modify the cultivation practices or improve the genetic potential of the plant to utilize resources more efficiently. The former option includes change in farming operations starting with tillage, weed control, mode of fertilizer application, irrigation etc. But this alone cannot bring about significant improvements, when genotypes are poor in input use efficiency. The second option is the alternate viable choice and sustainable approach. Therefore, breeders have an important role to play in this context. Hence, mining genes that enhances nutrient use or identifying donors that are efficient in their nutrient acquisition and utilization can be the breeding goals for research programs in coming decades.

Nutrient utilization by a plant involves multiple aspects and nutrient use efficiency is regarded as a polygenic trait determined by a plethora of activities including sensing, uptake, transport, and assimilation (Fan et al., 2017). This indicates that different nutrient uptake needs different specifications of plant morphology and genetic system. For example, the rice plant relies on its early shallow roots for phosphorus acquisition while longer roots serve better in nitrogen uptake at late growth stages. Such diverse variations in root morphology do exist in nature across cultivated varieties, wild species, and subspecies of rice. Evaluation of these resources would serve benefits to the cause of harnessing genes for enhancing nutrient use. The plethora of information available in genomic databases in form of marker data and SNPs linked to their respective traits makes the process less tedious. For instance, Phosphorus starvation tolerance 1 (*PSTOL1*),

a significant gene identified in the phosphorus-deficiency tolerance QTL, *phosphorus uptake 1* (*Pup1*) enhances early root growth and phosphorus uptake has been reported from the aus-type variety Kasalath under rain-fed and upland conditions (Gamuyao et al., 2012) and several QTL have been identified and reported for low P tolerance (Mahender et al., 2017). This chapter focuses on the major nutrients required for the healthy and productive growth of rice both in upland as well as lowland conditions and highlights the major breeding achievements and progress made in recent times.

2. NUTRIENTS WITH A MAJOR ROLE IN RICE PLANT DEVELOPMENT

2.1 Nitrogen

Nitrogen when compared to other nutrients is required immensely by the rice crop. It helps in root growth, tillering capacity, grain filling, *etc*. and at the same time, it also serves as an important component of biomolecules like nucleic acids, amino acids, chlorophyll, hormones and enzymes. It is highly mobile in the plants such that its recovery from deficiency symptoms is easily visualized. The application rate of nitrogen fertilizers has always been high as almost all soil types are N deficient. In such soils, the deficiency symptoms would be manifested by one of the followings: stunted plants and yellowish-green leaves with visibly reduced vegetative growth, in extreme conditions tiller number, as well as grain filling, is also hampered. Thus, the time of application and rate of application plays an important role in determining the N use efficiency. The time of N application in rice is synced with the functions regulated by Nitrogen in three phases. The initial dose is timed with the seedling establishment and development of tillers as a basal dose. The second one is at the time of panicle development/ initiation to enhance the number of grains per panicle. While the third and last dose aims at making the grains filled (Thenabadu, 1972). This grain filling phase is greatly influenced by the C: N ratio. Nitrogen Use Efficiency can be seen as the product of two separate functions; Nitrogen Uptake Efficiency and Nitrogen Utilization Efficiency (Das & J, 2015). The former is determined by how efficiently and actively roots undertake the nutrient uptake and then supply it to the shoots. The later is determined by the shoot and canopy activity. This utilization part is done by C fixation which in turn is dependent on N content in the plant biomass. This nitrogen from all over the plant is remobilized to the grains turning leaves and shoots dry (Das & J, 2015).Ultimately the canopy activity is reflected in yields and thus Nitrogen use efficiency is expressed as the ratio of yield produced to the amount of N available per unit of soil. Addressing the question of rate of application, inorganic source of nitrogen besides organic sources (such as farmyard manure) is the main form of fertilizer. The rule is to apply 40"50 kg N/

ha as inorganic fertilizer for every ton of additional grain yield compared to yield without N. It has been observed that rice crop under optimum conditions utilizes somewhere around 16 kg N per ton of grain yield (http://www.knowledgebank.irri.org/training/fact-sheets/nutrient-management/item/nitrogen).

Generally, the available forms of nitrogen in paddy fields are controlled by the existing soil conditions. Aerobic/upland conditions provide an oxidized environment where the predominant form is nitrate (NO_3^-), whereas the puddled anaerobic conditions provide the plant with ammonium (NH_4^+) form of nitrogen. These different available forms are peculiar to rice plants and thus the plant has evolved genes/ transporters for the uptake of the respective forms of nitrogen. The roots, assimilate a small portion of the nitrate from nitrogen and transport the other chunk to the shoots where it is reduced into ammonium form, while the nitrogen taken up in ammonium form is directly utilized by the plant cells or sink organs after converting into amino acids (Huang et al., 2018). This process of conversion is facilitated by the glutamine synthetase (GS)/glutamine-2-oxoglutarate amino transferase (GOGAT) cycle (Xu et al., 2012). Another gene *Dense and erect panicle 1 (DEP1)* that regulates the panicle architecture is also involved in nitrogen use efficiency in rice. The plants carrying the dep1 allele were sensitive to N at the vegetative stage, which increased the N uptake and utilization. The *DEP1* gene also aids in increasing grain number and grain filling. A study reported that NILs with the *DEP1* region exhibited erect panicles and an increased number of grains per panicle (Wang et al., 2020). A major QTL on chromosome 12, *Tolerance Of Nitrogen Deficiency 1 (TOND1)*, was identified in the *indica* cultivar Teqing that responded to low N in the soil (Zhang et al., 2015). The authors studied 75 *indica* and 75 *japonica* cultivars, of which only 41 *indica* cultivars harbored the gene while the rest did not have the gene. Another report suggested the main effect of QTL *qRDWN6*^{XB} on the long arm of chromosome 6 that impacts tolerance to N deficiency in *indica* rice variety XieqingzaoB. The QTL was fine mapped to a region of 52.3kb flanked by the markers ND-4 and RM19771 containing nine candidate genes (Anis et al., 2019). Of these nine genes, there was also a potassium transporter (LOC_Os06g15910) involved in the function of this QTL.

In rice, nitrate uptake is controlled by two different transporter families; nitrate/peptide transporter family (NPF) and Nitrate transporter 2 (NRT2/NAR2). Of these, the NPF family is the most explored with 80 genes reported but most of them are low-affinity transporters (the exception being *OsNPF6.5* which is a dual affinity nitrate transporter). This family of transporters display a wide range of activities with nitrogen that includes root-shoot transport (*OsNPF2.4*, *OsNPF2.2*), lateral root promotion (*OsNPF8.20*), panicle elongation (*OsNPF4.1*), and

increasing grain yield (*OsNPF8.20*) (Huang et al., 2018). The next group of transporters (NRT2/NPF2) comes under a high-affinity class as they play a role in uptake even in low concentrations of nitrates in the soil. Out of the five NRT genes discovered so far, *OsNRT2.3b* under high expression levels enhanced not only N, but also Fe and P uptake. Interestingly two other members; *OsNRT2.1*, and *OsNRT2.2* were expressed throughout the root system but couldn't alone work in nitrogen uptake but needed a partner protein like *OsNAR2.1*(Liu et al., 2014). Ammonium uptake in rice plants is facilitated by the ammonia transport protein (AMT)/ methyl ammonium (MEP)/rhesus (RH) super family. At least ten putative AMT genes have been operating in rice (three members in each group of *OsAMT1*, *OsAMT2*, and OsAMT3 and one member of *OsAMT4*). They are involved mostly in root uptake and root-shoot transport.

Therefore, nitrogen use efficiency estimates should take into the uptake, transport as well as assimilation efficiency of the rice genotype. Improving any of these can contribute to the nitrogen use efficiency of the rice significantly.

2.2 Phosphorus

Phosphorus, besides nitrogen, is another important nutrient required by rice plants. They are the important part of nucleic acid, energy conversion process, regulates root growth, proper tillering, flowering, and ripening. It has high mobility in the plant system but becomes immobile and inaccessible forming calcium salts and other complexes in the soil (Abel et al., 2002). The P deficient plants develop erect and dark green leaves, reduced tillering, delayed ripening, and unfilled grains (http://www.knowledgebank.irri.org/training/fact-sheets/nutrient-management/item/phosphorus). Phosphorus fertilizers are derived from rock phosphates that are non-renewable and are set to be exhausted in near future thus developing P use efficiency is of concern. Under optimal conditions, a rice plant utilizes around 6.4kg of P_2O_5 per ton of grain yield. As it gets fixed in the soil soon after its application, therefore the entire P dose is applied as the basal dose for the rice plant to acquire the required amount of the nutrient at the initial stage. Due to its immobility in the soil, P accumulation in the soil is limited to the shallow layers; hence the surface and subsurface roots have the responsibility of P uptake. This is evident in case of plants with well-developed surface and subsurface root systems to be more efficient in their P utilization (Ramaekers et al., 2010). *Phosphorus uptake 1* (*Pup1*),a major QTL enhances the early root growth and makes the plant more efficient to P utilization in deficient conditions (Gamuyao et al., 2012). This gene is present in one of India's mega variety Swarna, making it more adapted to P deficiency and high use efficiency in Indian soils that are mostly P deficient (Ali et al., 2018). Two new donors of the *PSTOL1* gene were identified among landraces and improved varieties; a landrace from Assam

(IC459373) and a short duration variety Shankar of Odisha. They performed at par with positive checks Kasalath and Dular (NRRI, 2019). Screening of landraces for *Pup1* indicated more P-uptake by the genotypes Bowdel, Lalsankari, Karni, N22, Tepiboro, Dular and Surjamukhi compared to Kasalath, taken as the positive check (Pandit et al., 2018). This shows that increasing root growth and root spread at the early stage of seedling growth can be an indirect trait contributing towards P use efficient rice plants. Rice plants can start phosphorus uptake actively as early as 2-3 days after germination, irrespective of the seed P reserves. Two such Phosphate transporters have been identified; *OsPHT1* and *OsPHT8* that play a major role in the reallocation of P from source to sink organs and help in the nutrient homeostasis in the growing embryo (Jia et al., 2011). In rice, 26 *OsPHT* genes have been identified to be involved in P transport, distributed across all the chromosomes except on chromosome 7 (Liu et al., 2011). Of these, 18 are localized in the plasma membrane, involved in the core transportation process across the membrane (Mahender et al., 2017).

Uptake of phosphorus is done by the transporters located in the root plasma membrane, *OsPHT6*, a transporter gene located in the root epidermis is a high-affinity transporter working well under deficient soil conditions (Ai et al., 2009). The P transporter genes have been classified into five groups based on their sequence and localization, namely *PHT1, PHT2, PHT3, PHT4*, and *PHT5* (Wang et al., 2017). Of these *PHT1* is mainly involved in the uptake of P from the soil, whereas the rest of the transporters are related to distribution and translocation of P within the cellular compartments(M³odziñska & Zboiñska, 2016). In rice, 13 transporter genes were reported under*PHT1*characteristically up regulated in P deprivation conditions (Nussaume et al., 2011). However, the other transporters such as *PHT2, PHT3,* and *PHT4* have not been well studied and understood. Studies on genetically modified rice crops have revealed that *OsPHT4* and *OsPHT6* also significantly increases 1000-grain weight and grain yield as compared to wild type under low Pi concentrations (Zhang et al., 2014, 2015). Interestingly, it was seen that phosphorus use efficiency is not a function of the Pi transporters alone, as P inefficient genotypes were able to uptake Pi from nutrient solution almost at the same rate as that of a tolerant genotype (Wissuwa, 2005). This indicates that the high expression of these transporters might be a part of the P starvation response instead of P starvation tolerance (Oono et al., 2013). Remobilization and allocation of the P after uptake can be a major problem, it was observed that over expression of *OsPT14* comparatively higher P accumulation in the top three leaves only (Wang et al., 2017). Thus, it is suggested that the focus now should be more on intracellular Pi transporters such that their remobilization and proper distribution can be facilitated. In addition, two sulphate transporters *OsSULTR3;3* and *OsSULTR3;4* have been reported to be involved in

P distribution in the plant cells (Zhao et al., 2016; Yamaji et al., 2017). A mutation study in these genes resulted in reduced grain P percentage (by 20%) while increasing the P content of the straws (Yamaji et al., 2017). This shows their function in the remobilization of nutrients during the grain filling stage at the later part of crop growth.

However, the fact that P fertilizer sources (rock phosphates) are non renewable and cannot be ignored. Thus, more emphasis has now been moved towards mycorrhizal associations that contribute to the efficient and simple solution to this problem. But as more area under rice is cultivated in the flooded ecosystem, it seems not suitable for fungal growth. Apart from this, the use of plant growth-promoting rhizobacteria would also help to supplement fertilizer application.

Arbuscular mycorrhiza (AM) is regarded as a root endosymbiosis, whereby the fungal entity accumulates nutrients (nitrogen and phosphorus) from the soil with the help of its network of mycelium while acquiring carbohydrates and lipid from the rice plant (Lefebvre, 2020). This association is initiated by the symbiotic signals from the AM fungi and is perceived by the plant receptor-like kinase *OsCERK1* of the host rice plants (Buendia et al., 2018). This gene not only allows the symbiotic association but also stimulates root branching. A study has shown that introgression of the *OsCERK1* allele from a wild source of rice increased the host plant's level of fungal establishment and colonization (Huang et al., 2020). The interesting view which has been put forward here is that, this trait in the discussion might have been subjected to negative selection from the initial times of rice breeding programs. It has also been reported that the *indica* cultivars of rice are rich with regard to this allele as compared to the *japonica* counterparts. Almost all these representations were from upland rice varieties grown in India (Sisaphaithong et al., 2017). As the AM fungi establishment is poor in transplanted system conditions of rice fields, they were mostly confined to the rice varieties cultivated in uplands.

Mycorrhizal role in N uptake has also been reported where the mycelium uptakes the nitrate ions, and converts it to arginine (Govindarajulu et al., 2005). This form is probably hydrolyzed into ammonia before transporting across to the host cells. The root-specific transporter gene *OsNPF4.5* comes into play at this point that helps in crossing the membranes delivering it to the host cells. A loss of function in this allele decreased the colonization level and efficiency in the rice plant (Wang et al., 2020). However, it is unclear that whether the nitrate can be directly crossed through the arbuscular membrane of the host plant cell without converting it into ammonium ions. Their role in phosphorus uptake is also a major area of growing interest. AM fungi can avail access to Pi beyond the depletion zone and can transport it back to the arbuscules for distances upto 25cm, thus

increases the total P uptake by the plant and also contribute to P use efficiency (Smith et al., 2003). Recent reports have highlighted two transporters; *ORYsa; Pht1; 13* and *ORYsa; Pht1; 11* involved in acquisition of P in association with AM fungi. *ORYsa; Pht1;11* was found to be only present in association with AM fungi colonized roots, while *ORYsa; Pht1;13* was active under all growth conditions (Glassop et al., 2007). Generally, AM fungi require dry conditions for their survival and are inhibited by anaerobic conditions, but it is reported that AM fungi maintain their functionality even under flooded conditions. AM fungi entered the field and established in the roots before flooding. They can even survive in the later stages of flooding (Nakagawa & Imaizumi-Anraku, 2015). A study on IR66 variety revealed that the AM marker genes maintained their higher level of expressions for P uptake under both aerobic as well as anaerobic conditions (Yang et al., 2012).

2.3 Micronutrients: Iron and Zinc

Iron is the most limiting nutrient for rice crops owing to its low solubility of the ferric form (oxidized ion) especially in aerobic/upland conditions (Zuo & Zhang, 2011). Iron is a structural component of the chlorophyll molecule, thus has a great role in photosynthesis. Any fluctuation in the level of Fe can lead to leaf damage, low assimilation, and ultimately reduction in yield. Even though it is abundant in the lithosphere, its bioavailability is limited, especially in alkaline soils and oxidized dry aerobic soils. The deficiency symptoms under such conditions can be easily visualized with its typical symptoms like interveinal chlorosis predominantly in younger leaves and reduced root growth. Under severe conditions, grain yield reduction as high as 50% has been observed and also complete crop failure at the vegetative stage itself (Mahender et al., 2019). Rice like any other *Poaceae* secretes root exudates or phytosiderophores to facilitate uptake of Fe from the rhizosphere. These phytosiderophores are released as a result of the action of nicotianamine synthase (NAS), nicotianamine aminotransferase (NAAT), and deoxymugineic acid synthase (DMAS) (Shojima et al., 1990). However, at the young seedling stage, due to lower levels of secretion of deoxymugineic acid, the root tips become chimeric and the epidermal cells become necrotic (Mori et al., 1991). These secretions help in forming soluble complexes with Fe in the rhizosphere, a form that can easily be taken up by roots. The roots pick up these Fe(III)-PS complexes from the soil with help of trans membrane transporters; *YELLOW STRIPE-LIKE PROTEINS* (*YSLs*) (Nozoye et al., 2011). Apart from *YSLs*, there are other transporters involved in the translocation such as *OsTOM1, OsIRT1, OsIRO2, OsIRT2*, and *OsNARMP1,* and genes, such as *OsNAS1-3, OsNAAT1, and OsDMAS1* (Mahender et al., 2019). These transporters efficiently deliver the iron to the vacuole for storage and they are made available only for vital life processes like respiration and photosynthesis.

Apart from these, during the initial establishment stages, rice seedling employs *IDEF1, IDEF2,* and *OsNAS2–3* and *OsNAAT1* during and after germination respectively (Mahender et al., 2019; Pradhan et al., 2020a). On the contrary under low pH conditions, iron gets reduced from its ferric (Fe^{3+}) form to its ferrous (Fe^{2+}) form, increasing its availability in acidic soils, this may lead to the problem of iron toxicity. Under such situations, root discoloration, reduced root growth, and bronzing of the leaves were reported. However, at such situations the excess of iron is retained in the root and which act aas a part of physiological root-based response to iron toxicity in rice.

Zinc is an essential plant nutrient involved in multiple biochemical pathways in rice plants, most importantly in the production of chlorophyll and maintaining cell membrane integrity. As obvious, zinc deficiency leads to defect in chlorophyll pigments and thereby the leaves develop characteristic discoloration. Also due to the loss in membrane integrity the cellular components lose their turgor pressure. Zinc is moderately mobile in the plant system while it is immobile in the soil. Under transplanted flooded conditions, the availability of zinc gets restricted to the plants. In such cases, the only option is to go for the external application of zinc fertilizers. Usually, zinc deficiency is not so common, but prevalent under neutral, calcareous soils and continuously flooded soils. The deficiency symptoms could be one or more of the following: stunted plants, brown spots on upper leaves, chaffy grains, delayed maturity, etc. The mechanism of zinc deficiency/ tolerance is not fully understood, but research activities are done so far suggest that it may be linked to the root development in rice plants (Rose et al., 2013). The root carries out zinc (Zn^{2+}) uptake with the help of Zn-regulated transporters (ZRT) and iron (Fe)-regulated transporter-like protein (IRT) that are included in the ZIP protein family (Guerinot, 2000). Several ZIP family proteins like *OsIRT1*, *OsIRT2*, *OsZIP1*, *OsZIP3*, *OsZIP4*, *OsZIP5*, *OsZIP7*, and *OsZIP8* have been reported in rice involved in zinc uptake from the soil, transport from the root to the shoots and the grains (Krithika & Balachandar, 2016; Pradhan et al., 2020b). Like Fe, response to Zn deficiency is also related to root exudates, phytosiderophores (MA). *ZmYS1*, a yellow stripe gene, has a range of specificity for metals including Zn, and thus helps in uptake and transport (Schaaf et al., 2004). However, some of these transporters also uptake cadmium into the plants which may be harmful from consumption point of view. But, if we talk of utilizing these transporters for bioremediation, then cleansing the soil from heavy metals would be easier.

3. IMPROVING NUTRIENT USE EFFICIENCY IN RICE BY SHUFFLING THEIR GENOME

3.1 Appraisal of germplasm for nutrient use efficiency

Rice landraces and wild relatives growing away from modern high input

agriculture form an untapped resource that harbor a range of genes which can help in enhancing nutrient use efficiency. Many genes have been identified that have contributed to nutrient stress from these sources. For instance, the discovery of the root enhancer gene *Pup1* from Kasalath and has led to the exploitation of *PSTOL1* (Gamuyao et al., 2012). This has greatly increased the phosphorus tolerance in varieties. There is a need to sequence and genotype the existing germplasm collections and then phenotype them using high throughput platforms that are getting more reliable over the past few years. An ongoing project in ICAR-NRRI, Cuttack in collaboration with Global Challenge Research Fund-South Asian Nitrogen Hub (GCRF-SANH) has been involved in screening a diverse group of *Aus* rice varieties, which have a characteristic feature of surviving under upland dry conditions. This population is known as Bengal and Assam Aus Panel (BAAP) and has representatives from landraces and improved lines. Currently, efforts are underway to identify promising lines for their role in nutrient use efficiency (nitrogen, iron, and phosphorus). The population has already been genotyped highlighting <"2 million informative SNPs which are to be utilized after phenotyping to identify superior lines with traits of interest. A study to screen and identify lines with low soil P tolerance was conducted among 98 germplasm lines collected from Northeastern India using *Pup1* associated molecular markers. This study reported a novel source of low P tolerance allele, completely devoid of *Pup1* from the landrace Wazuhophek (Swamy et al., 2019).A screening and evaluation study for low P tolerance allele was undertaken at ICAR-NRRI, Cuttack in a broad sample of three different *Oryza* species (O. *sativa, O. nivara,* and *O. rufipogon*). The study focused on the presence of the *PSTOL1* gene and related QTL along with the association of AM fungi via association mapping. The results highlighted a positive association between the root traits (volume and length) and the geometric top view area of the plant, this correlation can help in indirect selection for low P tolerance. The experiment also helped in the identification of alleles associated with low P tolerance in six different genotypes; IC459373, Chakhaoaumbi, AC100219, AC100062, Sekri, and Kumbhiphou. Screening for nitrogen tolerance gene *TOND1* was carried out across a mapping panel consisting of 173 rice landraces and improved lines under hydroponics. Out of these 173 lines, IRGC 78395-1, IRGC 28964-1, and IRGC 36826-1 were reported to be low N tolerant cultivars and validated with the presence of the *TOND1* gene (NRRI, 2019).

3.2 Identification of genomic regions involved in nutrient use efficiency

As already been mentioned in the previous sections that nutrient utilization is a complex trait consisting of many functions carried out together (uptake, translocation, remobilization, and assimilation), thus identifying the genes or the genomic regions dealing with these activities with respect to individual nutrients

is the basic step. This can be done using a variety of methods that include mutation and molecular mapping. In the case of nitrogen around 200 QTL have been identified in such programs (Neeraja et al., 2019). Software-based simulations, crop growth models, and high throughput phenotyping platforms can aid in this step. Development of gene-based or gene-linked markers for diagnosis of the gene of interest can be followed by sequencing-based trait mapping. In some cases, genome editing tools can also be utilized to edit the desired genes.

A recent study revealed a major QTL, *qRDWN6*^{XB} responsible for root dry weight under nitrogen deficiency has been fine mapped to a 52.3 kb region on chromosome 6 through chromosomal segment substitution. This region is also reported to be harboring a potassium transporter gene (Anis et al., 2019). An ongoing project in ICAR-NRRI, Cuttack in collaboration with Global Challenge Research Fund-South Asian Nitrogen Hub (GCRF-SANH) has been involved in screening and identifying superior genomic regions that are involved in NUE using the BAAP lines through association mapping.

3.3 Identifying efficient donors with superior haplotype

Once the genes or QTL involved with the nutrient are identified, the next step is to look out for genotypes harboring these respective genes. The genes are then transferred using Marker Assisted Backcross Schemes or in some cases with the help of a vector. Confirming these genes in the recipient and then using them in further trials would allow improving the existing varieties for better adaptation and higher yield.

Introgression of a gene from an elite variety has little or no linkage drag of unfavorable alleles as compared to that from a wild source that harbors many undesirable alleles linked to the gene of interest. An Indian mega variety, Swarna with the *Pup1*allele, has been quite popular and sustains a wide range of soil conditions (Ali et al., 2018). The identification of the *Pup1* allele for P deficiency tolerance in Swarna, an elite cultivated variety, eased the transfer of genes through the backcross scheme. ICAR-IIRR, Hyderabad used Swarna as the donor parent to transfer *PSTOL1 (Pup1)* gene to the background of another Indian mega variety, MTU1010 with the help of codominant CAPS markers (Anila et al., 2018). The selected lines after the backcrossing program showed better performance under phosphorus deficiency conditions along with better root growth and higher productive tillers. Similarly, they used Improved Samba Mahsuri (loaded with bacterial blight resistance genes) with the *Pup1*candidate gene *PSTOL1* (Swamy et al., 2020). They designed a new codominant marker K20-1-1 for foreground selection and used a set of 66 polymorphic SSR markers for background selection. The selected lines from BC_2F_6 were found to be well-performing under low soil P conditions. In order to comprehend the expression levels of P transporters

(*OsPHT1*) in presence of the gene *PSTOL1*, ICAR-NRRI, Cuttack studied six genotypes in an experiment designed with different levels of Phosphorus concentration (0.5-10.0 ppm) in a hydroponics system. The study aimed at highlighting three-way interactions (genotype (G) × phosphorus (P) × duration (D) at 14 and 28 days *via* the trait differences, for both shoot and roots. The results identified a new indigenous accession, IC459373, which had higher levels of expression of *PSTOL1*, as compared to the already reported low P tolerant genotypes; Dular and IR64-Pup1. Under deficient P, the transporter genes *OsPT1, OsPT2, OsPT6,* and *OsPT13* were reported to be more active in these tolerant lines.

Apart from the above-mentioned steps, some improvements and modifications in the breeding schemes and procedures can also aid in the process of genetic enhancement of the crop. For instance, instead of selecting traits directly on the basis of their phenotypic manifestation of traits, indirect selection can be adopted. ICAR-NRRI, Cuttack has been studying the genetic enhancement for P use efficiency and the results point out the correlation between the above-ground shoot/canopy geometry (traits like minimum enclosing circle, convex hull, and caliper length) to the root system architecture built below the ground. This sheds light on the importance of nondestructive image-based phenotyping in low P tolerant screening. The report also highlights the facts that shoot length, stem and root biomass, total root length, root volume, and root surface area could be utilized to supplement the screening in situations at the seedling stage where imaging technique is unavailable. A recent approach that has been gaining popularity among breeders; speed breeding or rapid generation advancement (RGA) can be utilized while designing breeding schemes such that the time and space utilized for growing and fixing the lines be minimized. This will enable breeders to carry out screening populations on large scale. However, this approach is limited to long-day crops and cannot be applied to photoperiod-sensitive crops. Looking at a different perspective, not only acquisition by transporters but research should also aim at identifying and then deciphering the action of transporters involved in remobilization and reallocation of nutrients within the cellular compartments of the plant cell. As the goal is to increase yield, NUE can only be beneficial when it is reflected in a genotype with better panicle features, large sink and improved grain filling ability in rice.

4. CONCLUSION

The existing trend of cultivation and consumption is both exhaustive and alarming in nature. The need is to maintain the growth trend, conserve and sustain the natural resources. To face the challenge of changing climate and depleting natural resources, especially plant breeders need to divert more attention in enhancing

nutrient utilization of the rice plant. This can either be done by improving plant traits by genotyping and high throughput phenotyping, improving specific plant morphologies (such as roots), or utilizing transporter genes for high uptake. Even though the traits in the discussion are polygenic and highly complex, MABC has been proved to be a successful and easy solution to the problem. Adopting a high throughput image-based phenotyping technique, multi trait-based data analysis, and holistic research effort involving various fields of science can ease the effort. These steps can ultimately lead to reduced consumption of chemical fertilizers and thereby conserve the existing resources both soil and rock reserves.

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Molecular breeding for genetic improvement of grain quality in rice

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SUMMARY

Quality breeding in rice has gained a major thrust with the increasing awareness of grain quality among its consumers. The quality expectations for rice widely vary among its consumers. This imparts the uniqueness to each rice quality breeding program, aiming for a different consumer group. The existing diversity in the germplasm of rice, serves to the diverse needs of quality among its consumers. Quality in rice refers to the physical and chemical properties before and after cooking. Pre-cooking qualities include the physical appearance of grain, its size, shape, color, uniformity in overall appearance and luster; Millers' qualities like, specific density of grain, hulling and milling recovery, head rice recovery etc. The post-cooking qualities include stickiness, volume expansion after cooking, and smoothness of surface after cooking, glossiness, and uniformity of particles after chewing, mouth feel after chewing, taste and flavor of whole grain after cooking etc. Looking into the wide acceptability of rice as staple, in cuisines and in desserts, the quality of this miracle grain can be discussed under four broad categories, its physical appearance, milling properties, sensory attributes (cooking and eating characteristics) and most importantly its nutritional profile. Nutritional quality includes protein, micronutrients and antioxidants. Individually each component serves very significant role in maintaining good health. But as the quality traits are quantitative in nature, and their expression is highly affected by environment, it is very important to understand the genetic nature of these traits before undertaking their genetic improvement for varietal development. The present chapter endeavors to compile the recent advances contributing to the understanding of molecular genetics of the quality traits and the underlying mechanism of expression of these traits. Advances in understanding of the underlying molecular genetics of these traits lead to the actual gene identification, its cloning and its functional validation. This paved the way towards development of functional markers for the quality traits that have been effectively utilized in varietal improvement programs. With the advancement of techniques in molecular genetics and instrumentation for precise phenotyping of quality traits, quality

breeding will achieve acceleration to serve the different target group of consumers through molecular breeding for these traits.

Keywords: Starch, aroma, waxy, vitamins, minerals, antioxidants, iron, zinc, phytic acid.

Abbreviations: QTL: Quantitative Trait Loci, SNPs: Single Nucleotide Polymorphisms, SSRs: Simple Sequence Repeat motifs, DNA: Deoxy-ribo Nucleic Acid, WU: Water uptake: Wx: Waxy, HRR: Head Rice Recovery

1. INTRODUCTION

Increasing consumers' awareness for quality and their purchasing power has accelerated the demand for quality products including foods, across the world. Till recent past, production of more food per unit area was the major target for the plant breeders. Up surging demand for quality foods that lead to better remuneration to its growers have compelled the plant breeders to pay more attention to the quality improvement of different plant products. Rice being one of the major staple food crops of the world, has not remained indifferent of this drive for quality, across the globe. Improvement of rice quality is rather more complex than yield due to the existing diversity in demand for rice quality among different types of consumers. To make it simple, rice quality is defined in terms of its stakeholders into millers' quality, consumers' quality and end-use quality. The consumers' quality is further complexed by the varying preferences depending on the geographic location, tradition, food habit of the consumer and the end use of rice.

Meeting the varying demands for different quality among its consumers, rice displays a huge diversity for different quality traits across the world. This provides an excellent scope for the plant breeders to work on the improvement of specific set of characters suiting to the demands of the target population. The genetic improvement of quality traits is further complexed by their quantitative nature. Phenotyping of the quality traits requires high cost, high throughput and instrumentation facility with specific skill to operate the instruments. Moreover, most of the protocols for estimation of the quality parameters are sample destructive in nature, restricting the early generation screening for quality traits in the breeding population. The quantitative nature of gene action for expression of quality traits makes them more prone to environmental variations. All these factors taken together make the work of quality rice breeders more challenging and therefore more rewarding.

Man's passion for quality produce has been existing since the beginning of agriculture. The ancient human being used to keep the best part of their harvest for generating the next crop. There has always been an unknown bias for quality.

As man became more aware of quality and found a proper market and remuneration for his quality produce, the drive for quality production was accelerated. The initial focus of rice quality was on aroma, grain size and stickiness due to their ease in phenotyping. With the advent of phenotyping facility in the form of high cost, high throughput instruments, many other traits including the nutritional parameters were also included. Although there are reports of many traditional genotypes with specific health benefits, least is known about the causal active ingredients in them. Revolution in the DNA marker technology during 1990's, immensely contributed to the identification of the genetic factors responsible for the expression of quality traits during 2000's. Advancement of phenotypic facility occurred later to the marker technology and therefore utilization of marker technology had to wait for the development of proper phenotyping facilities in case of quality traits. The initial mapping studies could land on to the specific regions of chromosomes in the form of QTL that reported to have major contribution in the phenotypic variation of specific quality traits. Huge cross talking and pleiotropic effect among the identified QTL were also reported. Later with the advancement in the sequencing technology and availability of huge sequence information at a very low cost, emerged the science of annotation and rice bioinformatics flourished. The online available gene annotation and function information could predict the probable genes within the earlier reported QTL responsible for the specific quality traits. Fine mapping also contributed immensely to generate information about the genetic factors responsible for quality traits. The transgenic technology could establish the function of the predicted functions of the isolated/ identified genes. The analysis of sequence information in different genotypes varying in quality (GBS: Genotyping by sequencing) for a specific region in the genome could identify the sequence variation leading to the change in phenotype. This could lead to the identification of functional sequence variations in the form of deletions, SNPs, or SNP haplotypes. The fine mapped QTL provided potential markers to undertake molecular breeding of these traits. The development of functional markers derived from the cloned genes helped in precise selection in breeding leading to precision breeding. Advancements in the molecular marker technology could overcome many limitations in breeding for these traits. The use of functional markers allowed the precise selection of genotypes in early stages of the crop and thereby reducing the duration required for breeding. The functional markers could also bypass the destructive methods of sample analysis and allowed to apply selection during early segregating generations *viz.*, $F_2$and F_3. Co-dominant markers could differentiate between the desirable homozygotes and heterozygotes during early stage of plant, allowing the selection of suitable genotypes for further crossing in the single generation, reducing the duration of breeding cycle. Marker assisted selection could overcome the limitations posed by environment in selection

of suitable genotypes, overcoming the selection slippage. Genes with pleiotropic affect could also be worked upon with the help of molecular markers.

All these interesting advancements in technology assisting and accelerating the studies and discoveries for deciphering the genetic factors controlling the quality traits in rice are presented in the following columns of the chapter. Wish the readers will enjoy reading the same.

2. GENES AND QTL FOR QUALITY TRAITS

2.1 Genes for the physical properties of grain

2.1.1 Length, breadth and width of the grain

Grain length, breadth and width decide the grain type and hence, the physical appearance of the rice grain. The preference for grain type hugely varies among the consumers across the globe. Huge variation in preference for grain type is observed to exist within a country. China, a major producer of rice in the world displays such variation in grain type preference by its consumers. People in North China prefer long slender grain type while short or medium bold grain type is the choice in South China. Similar variation in choice of grain type is observed in India as well. These niche variations in the preference of rice quality parameters make the study of its market dynamics and hence setting up of breeding objectives difficult.

A total of 47 QTL for grain length have been detected in different populations. Chromosome 3 harbors more QTL for grain length than the other chromosomes. Lou et al. (2009) reported a QTL for grain length on chromosome 3. Two QTL for grain length have been cloned using the technique of map-based cloning and seven QTL have been fine-mapped. *GRAIN SIZE 3 (GS3)* is the first QTL that has been map-based cloned for grain length. It plays major role in determining grain length and weight and minor role in grain width and thickness. Studies on the functional aspects of the gene revealed some interesting insights in its regulation and expression. The GS3 protein coded by the *GS3* gene is a negative regulator for grain size (Fan et al., 2006; 2009). The transmembrane Organ Size Regulator (OSR) domain in the N terminus of the GS3 protein, functions as a negative regulator of grain length. The deletion mutants for this domain result in the formation of long-grained rice. While the C-terminus domains of the same protein GS3 which comprises of a tumor necrosis factor receptor/nerve growth factor receptor (TNFR/NGFR)-like domain and a von Willebrand factor type C (VWFC) domain, act as positive regulators of grain length. The loss-of-function mutants of these domains lead to the development of very short grain (Mao et al., 2010; Takano-Kai et al., 2009).

The other major QTL for grain length that has been identified, cloned, and thoroughly studied is Grain length3 (*qGL3)*. The QTL has been identified in three independent mapping populations (Zhang et al., 2012; Hu et al., 2012; Qi et al., 2012) and putatively codes for the protein phosphatase with Kelch-like repeat domain (OsPPKL1). Transposition type base substitution mutation from Cytosine to Adenine which is very rare in nature than the transition kind of base substitution mutation, was found to be the cause for the long grain type. The transposition type base substitution leads to an aspartate-to-glutamate transition in the conserved AVLDT motif of the second Kelch domain in *OsPPKL1*, coded by *GL3* (Zhang et al., 2012; Hu et al., 2012; Qi et al., 2012). Hu et al. (2012) demonstrated the suitability of *qGL3* for marker-assisted selection in breeding programs by showing the genetic analysis of *qGL3* in near-isogenic lines (NIL) for *qGL3*.

A total of 48 QTL for grain width have been detected in different populations with more QTL on chromosome 3 and 5 (Figure 2) while four QTL have been map-based cloned. A major QTL between markers RM42 and C734b on chromosome 5 was found to be responsible for grain width (Tan et al., 2001). GRAIN WIDTH 2 (*GW2*) is a major QTL for rice grain width and weight. *GW2* encodes a RING-type E3 ubiquitin ligase (Song et al., 2007). GW2 negatively regulates cell division. The loss of *GW2* function results in an increase in cell number in the spikelet hull and acceleration of the grain-milk filling rate, thus enhancing grain width, weight, and yield. GRAIN WIDTH 5 (*GW5*) is another major QTL for seed width on chromosome 5 (*SW5*) (Wan et al., 2008; Weng et al., 2008; Shomura et al., 2008). A survey of *GW5/qSW5* polymorphisms in various rice landraces revealed that deletions in this gene may have played an important role in the selection of increased grain size from artificial and natural crossings during rice domestication (Shomura et al., 2008). Deployment of this QTL in rice variety Maudamani through marker-assisted backcrossing is likely to enhance the yield potential of this variety (Pandit et al., 2021).

GRAIN SIZE ON CHROMOSOME 5 (*GS5*) is a major QTL affecting grain width, grain filling, and grain weight (Li et al., 2011). It encodes a serine carboxypeptidase and functions as a positive regulator of grain size. GRAIN WIDTH 8 (*GW8*) is a major QTL affecting grain width and grain yield is also referred to as *OsSPL16.* A 10-bp deletion in the promoter region of *OsSPL16* has been found to be responsible for the slender grain trait in Basmati385 (Wang et al., 2012). *GS3*, *GL3*, *GW2*, and *GW5/qSW5* are negative regulators of grain size, while *GS5* and *GW8* are positive regulators. SMALL AND ROUND SEED (SRS) loci have been well reviewed in by Huang et al., (2013) that control grain size. Functional markers developed from these major genes and finely mapped QTL resources allowed breeders to efficiently manipulate grain size and shape. The

current progress made in the genetic basis of grain shape has been well summarized in a review article by Huang et al. (2013).

Besides quality, grain size and shape are yield attributing traits and are therefore, very important to study. A long, slender grain of rice is generally preferred by consumers in Southern China, the USA, and South and Southeast Asian countries, whereas consumers in Japan, Korea, and Northern China prefer short or round grain type (Huang et al., 2013). Grain length, grain width, length-to-width (grain shape) are the most stable properties of the variety, so they are highly heritable. The major genes controlling grain type have been map-based cloned with their functions well characterized, some are finely mapped. However, there are many genes with minor but regulatory effects are still waiting for their identification and characterization.

2.2 Genes for milling attributes

Milling properties refer to hulling recovery, milling recovery and head rice recovery after dehusking of paddy and polishing of the brown rice. These attributes largely affect the acceptance of the produce by the millers for milling and further processing. Therefore, the attributes are very significant for millers for better recovery of rice from paddy, as well as for the farmers to fetch higher prices for their produce from the millers. Several studies were undertaken to search for the genetic factors controlling the milling properties in rice. Some of the studies reported the markers linked to the milling properties to be utilized for the marker assisted breeding of these milling traits.

A total of 20 QTL related to the hulling/brown rice recovery and 19 QTL for milled rice recovery covering all chromosomes except chromosome 2 and chromosome 8, respectively have been identified. Three independent studies detected QTL for the milled rice recovery on chromosome 5 (Tan et al., 2001; Aluko et al., 2004; Zheng et al., 2007), but they do not share the same region. No QTL with major effect were identified for the milled rice recovery yet. Genetic studies revealed that brown rice recovery is associated with the grain shape and size. The existence of QTL-by-environment interaction in expression of the QTL for both brown rice and milled rice recovery was also indicated by Li et al. (2004).

A total of 34 QTL have been reported for HRR from ten mapping studies. Tan et al. (2001) identified a major effect QTL for HRR that coincided with a major QTL for grain length. This could indicate the dependence of HRR on grain length. A QTL on chromosome 3 have been identified across studies and genotypes, indicating the occurrence of a stable major gene in the region that needs further study (Li et al., 2004a; Dong et al., 2004; Aluko et al., 2004; Jiang et al., 2005; Lou et al., 2009). QTL on chromosomes 1, 5 and 6 are also detected by

at least three independent studies. Existence of environment and QTL-Environment-Interactions in the expression of HRR have been reported by Li et al. (2004) suggesting the role of multisite environment trials for selection of suitable genotypes andavoid the selection slippage.

2.3 Chalkiness of grain

Chalkiness affects the physical appearance of the rice grain and hence its acceptance among the consumers. Chalkiness occurs due to the development of large, complex starch granules that are round shaped and getloosely packed in the rice endosperm. Translucent endosperm consists of small sized, polyhedral starch granules that are placed singly and are tightly packed. Chalkiness is affected by both genetic and environmental factors. Environment at the time of grain filling plays a vital role in the development of chalky endosperm. Excessive heat, drought, or high wind at the time of grain filling is conducive for development of grain chalkiness. A genotype with translucent grains in general, may develop chalky endosperm if experiences stress during the grain filing stage. However, a chalky endosperm genotype will not develop translucent grain if grown under congenial environment. Genes involved in the starch biosynthesis pathway, development of starch granule structure by folding and branching, and grain filling affects the chalkiness of the endosperm. These genes include starch branching enzyme IIb (BEIIb), branching enzyme I (BEI), starch synthase IIIa (SSIIIa), floury and sugary genes, genetic male-sterility gene *ms-h* (Woo et al., 2008) that has pleotropic effect on chalky endosperm development, grain incomplete filling 1 (*gif1*) gene (Wang et al., 2008a), floury endosperm-4 (*flo4*) gene etc. The *flo4-2* and *flo4-3* also show white-core endosperm phenotype. Several allelic variations have been reported for the *Flo* locus. The *flo4-2* and *flo4-3* showed white-core endosperm phenotype. Floury endosperm-4 (*flo4*) rice mutant with a floury-white endosperm was generated by T-DNA insertion into the fifth intron of the OsPPDKB gene encoding pyruvate orthophosphate dikinase (PPDK) (Kang et al., 2005). Floury-5 (flo-5) mutation caused by T-DNA insertion in the SSIIIa gene exhibits a white-core floury endosperm in the innermost endosperm (Qiao et al., 2010). *FLO(a)* gene has been isolated via map-based cloning approach and was predicted to encode the tetratricopeptide repeat domain containing protein, OsTPR. It is envisaged that OsTPR motifs may play significant role in rice starch biosynthesis that causes the formation of grain chalkiness. Two QTL have been fine mapped. *qPGWC-8* is a major QTL for the percentage of grains with white chalkiness in the interval G1149-R727 on chromosome 8 which was identified using a chromosome segment substitution line (CSSL). Guo et al. (2011) narrowed down the location of this QTL to a 142 kb region between Indel markers 8G-7 and 8G-9. *qPGWC-8* accounted for 50.9% of the difference in PGWC between the parents. The markers tightly linked to *qPGWC-8* and *qPGWC-7* facilitates cloning of the gene underlying the

QTL and is of value for marker-assisted selection for endosperm texture. However, it is still far away from clear understanding of the mechanism of formation for the grain chalkiness. First, the QTL mapping results show low coherence in different genetic populations, suggesting the role of many minor QTL for chalkiness in different rice germplasm that we do not know. Second, in addition to the major genes or QTL we have known, how their interactions with each other, and with the major genes for amylose and protein synthesis (Liu et al., 2010; Zheng et al., 2012) that may affect chalkiness are unknown. Third, effect of environment on the formation of chalkiness is well known, but how its effect on the gene expression that leads to the formation of chalkiness is largely unknown.

2.3 Genes for biochemical properties of grain quality

2.3.1 Genes for aroma

The aroma of cooked rice contributes to consumer sensory acceptance of rice. The aromatic compound 2- acetyl-1-pyrroline (2-AP) is the primary component of the popcorn like smell of aromatic rice. Fragrance (fgr) is a recessive trait that is controlled by a major gene on chromosome 8 (Lorieux et al., 1996; Jin et al., 2003). Bradbury et al. (2005a; 2005b) reported that the *badh2* gene could most likely be the *fgr* gene since it has an 8-bp deletion which encodes putative betaine aldehyde dehydrogenase 2 (BADH2), and developed molecular markers for fragrance genotyping. Shi et al. (2008) found a novel null badh2 allele (badh2-E2), which has a sequence identical to that of the *Badh2* allele in exon 7, but with a 7-bp deletion in exon 2. By map-based cloning strategy, Chen et al. (2008c) confirmed that the full-length BADH2 protein encoded by Badh2 renders rice nonfragrant by inhibiting biosynthesis of 2-acetyl-1-pyrroline (2AP), a potent flavor component in rice fragrance. Functional markers derived from *fgr* are sufficient to carry out molecular marker assisted breeding to improve the sensory quality of rice (Shi et al., 2008; Chen et al., 2008c; Jin et al., 2010). So far as we are aware, there is no genetic report on the other sensory characteristics of rice.

2.3.2 Genes for starch and its quality

The molecular basis of starch biosynthesis has been well understood in the past two decades. Significant progress was attained in understanding the genetic basis of starch synthesis and its effect on the eating and cooking quality of rice. Extensive studies on structure and function of genes lead to the characterization of 14 different alleles of the major locus controlling starch biosynthesis in rice, known as the *Waxy* locus, designated as *Wx*. The starch biosynthesis, molecular structure of starch and its branching have been found to play very significant role in deciding the overall eating and cooking quality of rice. Starch is a macromolecule made up of glucose subunits bind up in alpha bonding. Depending on the intensity of branching of the macromolecule starch has two main components, amylose and

amylopectin and amylopectin like amylose. Amyloses are highly branched starch molecules while amylopectin are long chained starch molecules with small branches. A few are amylose like Amylopectin molecules that are characterized by their high branching but long molecules and short branch sizes. The amylose like amylopectin is reported to play significant role in controlling the eating and cooking quality of rice.

Granule bound starch synthetase 1 (GBSSI) enzyme is known to play a major role in the amylose synthesis while starch synthetase (SS), branching (BE) and de-branching enzymes (DBE) are known to play significant role in the synthesis of amylopectin. The expression of GBSSI is regulated by the *Wx* (waxy) locus that in turn is responsible for the existing variation in amylose content, gel consistency and RVA pasting viscosity. The enzyme *SSIIa* is responsible for gelatinization temperature, thermal properties, and amylopectin structure (Bao, 2012). Map based cloning of *qGC-6* lead to the discovery of the *Wx* locus (Su et al., 2011). This locus was later found to be a major player in deciding the quantity and quality of the starch. The structural analysis of the locus through GBS in different genotypes varying in amylose content, lead to the discovery of three sequence alterations resulting in functional changes in the phenotype. Five functional markers have been developed using the three functional sequence variations identified. These markers are now being used in improving the cooking and eating quality of popular varieties as amylose content is known to be a major player in deciding the cooking and eating quality of rice. These three functional sequence variations include a micro satellite repeat motif $(CT)_n$ (Ayres et al., 1997; Bligh et al., 1995) located at 55 bp upstream of the putative 52 -leader intron splice site, a 23bp insertion/ deletion sequence (Inukai et al., 2000; Wanchana et al., 2003; Teng et al., 2012) and a single nucleotide transversion type base substitution leading to change in the amino acid sequence and therefore leading to functional change in the enzyme activity. Higher frequency of unequal crossing over or replication slippage might be the reason for higher frequency of alleles, ranging from 8-22 at $(CT)_n$ in different studies germplasm (Ayres et al. 1997; Bergman et al., 2001; Bao et al., 2006a; Chen et al., 2008a; Bao et al., 2002a; Han et al., 2004). The T SNP allele is associated with the waxy, low or intermediate amylose content. The T allele reduces the efficiency of Wx- pre-mRNA processing leading to lower enzyme activity and lower amylose content. G/T SNP could explain 80% to 90% (Aryes et al., 1997, Bao et al., 2006a) of the total observed variation in AAC in the nonwaxy rice accessions. Since amylose content is not the ultimate determinant of cooking and eating quality of rice, and so is the *Wx* locus.

Amylose comprises 0 to 30% of the starch present in the grain. The rest of starch is amylopectin or amylose-amylopectin intermediates. In waxy rice amylose content is negligible and therefore almost the total of starch comprises of

amylopectin. Therefore, role of amylopectin shall not be demeaned in deciding the taste, cooking, and eating quality of rice.Very little knowledge has been generated for the biosyntheis of this component. Enzymes such as phosphorylase (Pho1), SS, SBE, or DBE, AG Pase, ADP glucose pyrophosphorylase, isoamylase (ISA) and pullulanase (PUL) have been reported to play role in the biosynthesis of amylopectin, gelatinization temperature and in deciding the starch pasting properties (Bao et al., 2002, Tian et al., 2009, Yan et al., 2011). The *Wx* locus has been found to influence the gel consistency that is the softness of the gel after gelatinization. A cytosine to thymidine base substitution at the tenth exon of *Wx* locus was found to correlate with the gel consistency. A cytosine at the locus leads to, soft, viscous gels, giving a soft texture when the rice is cooked. While SNP allele T (Thymidine) gives a short, firm gel, with a firm texture of the freshly cooked rice that is little changed over storage.

The *SSIIa* locus on chromosome 6 is found to have a major role in deciding gelatinization temperature and amylopectin structure (Gao et al., 2003). Functional SNPs have been reported in case of *SSIIa* locus. The first one is a G to C transversion at 264 bp in exon 1 of AY423717, leading to glutamate to aspartate substitution. The second is G to A transition at 3799 bp, where glycine is replaced by serine. The third one is again a G to A transition at 4198 bp, where valine replaced by methionine. The fourth one is GC/TT substitution at 4330 bp leading to replacement of leucine by phenylalanine. The GC/TT polymorphism at the fourth locus alone accounts for the 62.4% of the total variation available for pasting temperature that is associated with the gelatinization temperature in rice. Genotypes with GC allele at the fourth locus are reported to be associated with low GT phenotype. However, most of the outliers could be explained based on the allele at the third locus. An A at the third locus with a GC at the fourth leads to low GT. The third locus is crucial for *SSIIa* activity. An A at the locus makes the *SSIIa* inactive independent of the allele at 4229/4330 bp (GC/TT). However, an A at the third locus has been found to be a rare event in many studies(Chen et al., 2003, Umemoto et al., 2004, Waters et al., 2005, Bao et al., 2006b, Cuevas et al., 2010).The genetic control of intermediate gelatinization temperature has yet not been well deciphered. Besides amylose content, amylopectin content, gel consistency, gelatinization temperature and starch pasting parameters, there are a few other but important characters that decide the cooking and eating quality of rice. These include water absorption, cooking time, volume expansion and cooked rice elongation. Factors determining the cooking and eating quality of waxy rice are complex, because the GBSS is not active in waxy rice. It is expected that genes other than *Wx* to control the genetic basis of pasting and thermal properties in waxy rice. Waxy rice with the SBE-A allele have higher peak viscosity (PV), hot paste viscosity (HPV) and cold paste viscosity (CPV) than those with

other alleles, and those with the SSS-B allele have higher HPV and CPV than other alleles (Bao et al., 2002a). The pullulanase enzyme coding gene plays an important role in control of PV (peak viscosity), HPV (hot paste viscosity) and CPV (cold paste viscosisty), breakdown viscosity, peak time, and pasting temperature (PT) in glutinous rice (Yan et al., 2011). Since taste, cooking and eating quality of rice is most important to gain consumers' preference, extensive studies are being undertaken to solve this puzzle.

2.4. Genes and QTL for Nutritional quality in rice

Enriching rice with nutritional compounds is important to combat the problem of malnutrition and hidden hunger among the economically backward population. Rice has been a staple food for majority of such population across the globe. Because of this, rice is also popularly known as the poor man's crop. Therefore, enriching rice with the essential nutrients will always provide a good supplement to the population remaining deprived of a nutritionally balanced diet. Biofortification has always been recommended as better than the external fortification of food grains for better health and hygiene. Being quantitative in nature, the expression of nutritional traits is highly affected by environment. QTL controlling the nutritional quality have been well reviewed by Mahender et al. (2016) for use in grain improvement programs.

2.4.1 Protein content and quality

Among all other nutrients, protein content has always remained an area of focus for improvement in rice. Enrichment of protein content in rice will be very significant in combating the protein-energy malnutrition in the population. Since rice is the lowest protein containing crop among all other cereals, genetic improvement for grain protein content has always attracted the rice breeders. A total of 43 QTL have been identified covering all 12 chromosomes. Zhang et al. (2008) detected 2, 4, 3 and 4 QTL for protein fractions, albumin, globulin, prolamin and glutelin, respectively. Wang et al. (2008c) identified 18 chromosomal regions for 19 individual amino acids, one of which at the bottom of chromosome 1 is a relatively strong QTL cluster, consisting of up to 19 individual QTL. Hu et al. (2009) identified a total of 12 QTL for individual amino acid content and total amino acid content on chromosomes 1, 4, 6, 7 and 11. A QTL cluster on chromosome 1 was associated with the content of eight amino acids. All the results could indicate the existence of a region on chromosome1 that affects protein content and quality and could be significant for marker assisted breeding.Protein content and quality being highly affected by environment and genotype-environment interaction, identification of QTL expressing stably across environment and genotypes is extremely difficult. Chattopadhyay et al. (2019) identified three stable QTL for protein content in rice using BC_3F_4 mapping population. The mapping population was developed using grain protein donor, ARC10075 and a high-

yielding cultivar, Naveen. One QTL was for GPC (*qGPC1.1*) and the other two for single grain protein content (*qSGPC2*.1, *qSGPC7*.1) that were found to be stably expressed over environment explaining 13%, 14% and 7.8% of the phenotypic variance, respectively. QTL, *qPC3.1, qPC5.1* and *qPC9.1* controlling grain protein content were detected while four QTL *qPC3, QPC8, qPC6.1* and *qPC12.1* were validated in an association mapping study (Pradhan et al., 2019). High-protein lines reported higher glutelin content with the introgression of *qGPC1.1* in telomeric region of short arm of chromosome 1. This was supported by the postulation of probable candidate gene inside the QTL region encoding glutelin family proteins. Such stably expressed QTL needs to be targeted for fine mapping, structural and functional studies of the genes involved and development of useful functional markers for undertaking breeding for protein content and protein quality enrichment in rice.

2.4.2 Fat content

Fat content is important for both the eating quality and nutritional quality. Enrichment of fat content is also very significant in combating the Protein energy malnutrition. Fat content and its quality are also important to decide the storability of rice. Nearly 48 QTL for fat content have been reported so far. Chromosome 1, 3 and 6 harbors more QTL than other chromosomes. Liu et al. (2009) reported 14 QTL for crude fat content in brown rice. One of them on which is a major QTL, *qCFC5*, locating on chromosome 5, which have been detected simultaneously among three populations. Shen et al. (2012) characterized two stably expressed QTL on chromosome 7, and they were detected in all three environments and were further confirmed by additional lines across six environments. The stably expressed QTL and major QTL are suitable candidates for the improvement of fat content via marker assisted breeding. Dynamic expression of QTL for fat content during grain filling was detected by Wang et al. (2008b). Eleven unconditional QTL and 10 conditional QTL for fat content were identified with more QTL expressed in the early developmental stages. The results suggested that accumulation of fat was governed by time-dependent gene expression. Ying et al. (2012) identified QTL for fatty acid composition, and 29 associated QTL were identified throughout the rice genome, except chromosomes 9 and 10.

2.5. Minerals, phenolics and other nutritional compounds in rice grain

A strong QTL for oleic (18:1) and linoleic (18:2) acid is associated with a gene encoding acyl–CoA:diacylglycerol acyltransferase, while another one for palmitic acid (16:0) is possibly associated with the acyl–ACP thioesterase gene.

2.5.1. Minerals

Stangoulis et al. (2007) mapped the QTL for inorganic phosphorus (P), total P, Fe, Zn, Cu and Mn concentrations. Norton et al. (2010) mapped 41 QTL for the

concentration of 17 elements in rice grain. Du et al. (2013) identified 23 and 9 QTL for Ca, Fe, K, Mg, Mn, P, and Zn contents in brown rice in two environments of China, Lingshui of Hainan and Hangzhou of Zhejiang, respectively. Only 2 QTL for Mg accumulation have been detected in both environments, indicating that mineral accumulation QTL in rice grains are largely environment-dependent. Garcia-Oliveira et al. (2009) identified 31 putative QTL for Fe, Zn, Mn, Cu, Ca, Mg, P and K contents with introgression lines derived from a cross between an elite *indica* cultivar Teqing and the wild rice (*Oryza*). It was found that wild rice contributed favorable alleles for most of the QTL (26 QTL), and chromosomes 1, 9 and 12 exhibited 14 QTL (45%) for these traits. Pradhan et al. (2020) detected QTL *qFe3.3* and *qFe7.3* for grain Fe and *qZn2.2*, *qZn8.3* and *qZn12.3* for Zn content reported 1sttime. In addition they validated *qFe1.1*, *qFe3.1*, *qFe5.1, qFe7.1, qFe8.1, qZn6, qZn7 and gRMm9–1* for grain Fe-Zn content using association mapping. Phytic acid (myo-inositol 1, 2, 3, 4, 5, 6-hexakisphosphate) in rice grain may form complexes with mineral ions, such as Fe, Zn and Ca, leading to be low bioavailability of minerals to humans. A set of low phytic acid rice mutant lines with the aim of increasing the bioavailability of the minerals of rice (Liu et al., 2007) have been isolated. Functional markers have been developed from some mutants (Zhao et al., 2008; Tan et al., 2013), and candidate genes such as multi-drug resistance-associated protein ABC transporter gene 5 (Xu et al., 2009) have been revealed. These mutant and markers tagged for the mutation may help to develop new rice with increased mineral bioavailability.

2.5.2 Phenolics

Jin et al. (2009) found via linkage mapping that phenolic content, flavonoid content, and antioxidant capacity were individually controlled by three QTL. Only one QTL on chromosome 2 was shared by phenolic content and flavonoid content. Shao et al. (2011) identified QTL for these traits via association mapping using a diverse set of rice germplasm including red rice and black rice. Four, six and six QTL were found associated with phenolic content, flavonoid content, and antioxidant capacity, respectively. Among them, four QTL for phenolic content were also shared for other two traits. Ra (i.e.Prp-b for purple pericarp) and Rc (brown pericarp and seed coat) were main-effect loci for rice grain color and nutritional quality traits. Association mapping for the traits of the 361 white or non-pigmented rice accessions (i.e. excluding the red and black rice) revealed marker (RM346) is associated with phenolic content. Pigmented rice accumulates anthocyanins (black rice) and proanthocyanidin (red rice), which are benefit to human health. Genetically, the pericarp color of red rice was controlled by two complementary genes, Rc (brown pericarp) on chromosome 7 and Rd (red pericarp) on Genes and QTL for Rice Grain Quality Improvement http://dx.doi.org/10.5772/56621 257chromosome 1. When present together, these loci produce

red seed color. *Rc* in the absence of *Rd* produces brown seeds, whereas Rd alone has no phenotype (Sweeney et al., 2006; Furukawa et al., 2007). A natural mutation in rc has reverted brown pericarp to red pericarp and resulted in a new, dominant, wild-type allele, Rc-g (Brooks et al., 2008). The color of dark purple pericarp was also controlled by two complementary genes, *Pb* and *Pp*, located on chromosome 4 and 1, respectively (Wang et al., 2009). Wang and Shu (2007) mapped *Pb* gene and suggested that this gene may be *Ra* gene. Markers for these genes may be useful for pigmented rice breeding, especially useful if new rice expects to accumulate both anthocyanins and proanthocyanidin.

3.1. Marker assisted selection

QTL underlying natural variation in grain quality have been widely explored, however, only few of them have been applied in current rice breeding programs. To the best of our knowledge, most of reports in terms of improving grain quality simply mean to improve the eating and cooking quality. The most useful genes are *Wx*, *SSIIa*, and *fragrance*. Functional markers developed from GS3 are also available for grain length improvement (Wang et al., 2011). There are two strategies to perform MAS in the breeding program. One is to improve the grain quality for the rice with high yield potential or high resistance to abiotic or biotic stresses, but with low quality. This is referred to foreground selection, which means that selection of a trait introduced from donor genotype which include grain quality by using molecular marker. Foreground selection is particularly useful for traits that need laborious or time-consuming phenotypic screening procedures, such as grain quality traits. The other is to select thebackground traits using markers which include yield potential and high resistance in good quality rice, such as basmati or jasmine rice. This is referred to background selection. The markers for grain quality are used as background selection, which is to avoid the loss of good quality traits during introduction of the other traits.

3.1.1. Combining fragrance with starch quality

Wx, fgr and *SSIIa* impart Low quality of hybrid rice in China is mainly owing to the poor grain quality in the maintainer lines. One of good ways is to improve the quality of maintainer line by MAS. Some important maintainer line, such as Zhenshan 97B (Zhou et al., 2003; Liu et al., 2006), Longtefu B (Liu et al., 2006), and II32B (Jin et al., 2010), G46B (Gao et al., 2009) have been the target of transferring the *Wx* allele conferring lower amylose content. The new hybrid rice derived from the improved maintainer line and restorer line is expected to have better quality. Furthermore, quality improvement of *Wx* gene marker through MAS integrated with the conventional rice has been reported (Yi et al., 2009; Jantaboon et al., 2011; Jairin et al., 2009). Consumers generally prefer fragrant rice to non-fragrant rice. Functional markers for fgr have been developed and

successively used to transfer this gene from fragrance rice to the target non-fragrance rice (Yi et al., 2009; Jin et al., 2010; Salgotra et al., 2012; Jantaboon et al., 2011). *SSIIa* is responsible for the variation of gelatinization temperature; the functional markers for *SSIIa* have been developed and used in MAS to improve the cooking quality (Jin et al., 2010; Jantaboon et al., 2011; Lu et al., 2010).

3.1.2. Combining grain quality with other traits:

Breeding approaches suggested for not only one trait, but all the traits for the development of a new variety. In addition to grain quality traits, yield and other agronomic or resistance traits are also very important. For those rice cultivars already have good quality, the objective of MAS is to combine the important quality traits with other traits. There are special cases for basmati and jasmine rices which have premium grain quality, and have been widely accepted by consumers worldwide. MAS has been carried out to introduce bacterial blight resistance (Pandey et al., 2013; Win et al., 2012), blast resistance (Singh et al., 2012), brown planthopper resistance (Jairin et al., 2009), submergence tolerance (Jantaboon et al., 2011) and plant stature (Pandey et al., 2013) genes into the basmati or jasmine rices.

4. TRANSGENIC ENGINEERING

The advantage of MAS is that abundant molecular markers are available for rice and many traits have been tagged with molecular markers. However, the disadvantage is that MAS is only effective when the target traits exist in rice germplasm, and becomes void when the traits of interest are not present in the rice germplasm. In this case, transgenic engineering is useful, which could introduce the new traits into rice by transferring the target gene from other species. Expression of exotic gene in rice could produce the target trait. Genes and QTL for Rice Grain Quality Improvement has some successful examples to introduce new nutrient traits into rice grain, such as vitamine A (Va), that confers rice high nutritional and increased benefit to human health (http://dx.doi.org/10.5772/56621).

4.1. Resistant starch

Consumption of resistant starch enriched foods is associated with decrease in the postprandial glycaemic and insulinaemic responses, accompanied by the production of fermentation related gases in the large bowel. A high-amylose transgenic rice line modified by antisense RNA inhibition of starch branching enzymes showed 8.05% of resistant starch content, which was to decrease the postprandial glycaemic and insulinaemic responses and promoted fermentation-related production of H2 in the large bowel of young and healthy adults who consumed the resistant starch-enriched rice meal (Li et al., 2009).

4.2Protein content

Protein Expression of a gene encoding a precursor polypeptide of sesame 2S albumin, a sulfur-rich seed storage protein in transgenic rice plants results in the improvement of the nutritive value of rice; the crude protein content in rice grains was increased by 0.64-3.54%, and the methionine and cysteine contents of these transgenic rice grains were respectively elevated by 29-76% and 31-75% compared with those of wild-type rice grains (Lee et al., 2003). Over-expression of aspartate aminotransferase genes in rice results in altered nitrogen metabolism and increased amino acid content and protein contents in seeds (Zhou et al., 2009).

4.3Vitamin A content

Vitamin A deficiency has been linked to night blindness, corneal scarring and permanent blindness. Vitamin A deficiency increases infant mortality rates and the incidence and severity of infectious diseases. Carotenoids, a precursor of Vitamin A, is an important lipid-soluble antioxidants in photosynthetic tissues, which are known to be completely absent in rice endosperm. The entire â-carotene biosynthetic pathway in rice endosperm has been introduced into rice by transformation of plant phytoene synthase, Erwiniauredovora carotene desaturase, and lycopene β-cyclase genes via Agrobacterium-mediated transformation. The transgenic rice, Golden Rice 1, can accumulate a maximal level of 1.6 µg/g total carotene in the endosperm. Insertion of the phytoene synthetase gene from maize and the carotene desaturase gene from *Erwinia uredovora* into rice resulted in the greatest accumulation of total carotenoids and β–carotene. Golden Rice 2 contains as much as 37 µg total carotenoids per gram of dry weight of grain, of which 31 µg/g is β–carotene (Paine et al., 2005).

4.4. Folate

Folates are B vitamins (vitamin B9). Humans cannot synthesize folates and have to absorb them from the diet, with plants usually being the main dietary sources. Folates play roles in the prevention of neural tube defects and in reducing the risk of cardiovascular disease, colon cancer, and neuropsychiatric disorders. In the United States, folic acid is added to refined cereals and grain products; these products are major contributors to total folate intake. Rice is 260 Rice - Germplasm, Genetics and Improvementa poor source of folates (vitamin B9). Overexpressing two *Arabidopsis thaliana* genes of the pterin and para-aminobenzoate branches of the folate biosynthetic pathway, Storozhenko (2007) obtained transgenic rice with a maximal folate content enhancement as high as 100 times above wild type, with 100 g of polished raw grains containing up to four times the adult daily folate requirement.

4.5. Minerals (Fe)

Iron deficiency is the most widespread micronutrient deficiency world-wide that afflicts an estimated 30% of the world population, especially where vegetable-based diets are the primary food source. Expression of the soybean ferritin gene (Goto et al., 1999) or pea ferritin gene (Ye et al., 2007) in rice produced seeds with greater Fe contents. Especially, Vasconcelos et al. (2003) showed that expression of the soybean ferritin gene under the control of the glutelin promoter in rice has proven to be effective in enhancing grain nutritional levels, not only in brown grains but also in polished grains. Expression of a thermo tolerant phytase gene from *Aspergillus fumigatus* in rice endosperm is expected to decrease the phytic acid and increase iron bioavailability (Lucca et al., 2001).

4.6. Flavonoids

Flavonoids are lacking in the endosperm of rice. Expression of maize C1 and R-S regulatory genes driven by an endosperm specific promoter of a rice prolamin gene in rice grain resulted in dark brown pericarp of the C1/R-S homozygous lines, and the major flavonoids, dihydro quercetin (taxifolin), dihydroisorhamnetin (32 -O-methyl taxifolin) and 32 -O-methyl quercetin were identified in the rice grain (Shin et al., 2006). These rice lines have the potential to develop as a novel variety that can produce various flavonoids in its endosperm.

4.7. Serotonin

Serotonin derivatives such as p-coumaroylserotonin and feruloylserotonin, a family of plant polyphenol compounds, play roles in an array of biological activities including antioxidative activity, but neither their production nor identification has been reported in crop plants. Transgenic rice expressing the pepper hydroxycinnamoyl-CoA: serotonin N-(hydroxycinna moyl) transferase gene produced on average 274 ng/g seed weight which was nine-fold higher than wild-type (30 ng/g seed weight) (Kang et al., 2005). Chemical treatments such as transcinnamic acid and tyramine increased the serotonin derivatives contents by two- to three fold in both wild-type and transgenic rice. The transgenic rice had higher radical scavenging activities than that of wild-type, suggesting that neutraceutical serotonin derivative could be enriched by transgenic engineering (Kang et al., 2005).

4.8. Coenzyme Q

Coenzyme Q (CoQ), also called ubiquinone, is an electron transfer molecule in the respiratory chain. CoQ is also a lipid-soluble antioxidant. Most cereal crops produce mainly CoQ9, which has nine isoprene units, whereas humans produce mainly CoQ10, with 10 isoprene units. Genes and QTL for Rice Grain Quality

Improvement http://dx.doi.org/10.5772/56621 261CoQ10 is a very popular food supplement. Takahashi et al. (2009) produced CoQ10-enriched rice plants by introduction of the gene for decaprenyl diphosphate synthase. In CoQ10- enriched rice plants, seed CoQ10 content per weight was increased to up to 10 times that of wild-type rice, but its level is still insufficient for practical use. Combination of the transgene with giant embryo mutant lines produced giant embryo line-type CoQ10-enriched rice with seed CoQ10 content per weight increased to up to 1.4-1.8 times. It was found that CoQ was preferentially accumulated in bran and germ of rice seed.

5. FUTURE DIRECTIONS

Great progress has been achieved in our understanding of the genetic and molecular basis of grain quality in rice. This is especially true for grain appearance and grain shape, since they are not only linked with grain quality, but also with grain yield, and therefore, considered as an important trait. Cooking and eating quality has a strong relation with starch biosynthesis pathway which has been well understood. Markers derived from the starch biosynthesis related genes have been widely applied in MAS. However, there are four major problem areas that challenge the researchers working on molecular genetics of grain quality.

5.1. Functional genes for milling quality and chalkiness

Genetic understanding of milling quality is quite poor as only limited numbers of QTL have been detected, and no QTL has been finely mapped or cloned. In order to achieve a better understanding and deeper insight into the area of milling quality there is a need of rapid and accurate analytical tools for measuring the trait, finer mapping and dissection of QTL with large effect and molecular analysis of mutants that are induced by T-DNA insertion to characterize the genes responsible for milling quality.For grain chalkiness, two finely mapped QTL await further characterization, and transcrip tome for chalkiness formation during seed development have been described (Yamakawa et al., 2007; Liu et al., 2010). It looks optimism to see more progress from this area.

5.2. Molecular genetics studies for nutritional quality

Nutrition quality of rice will be a new area for further research because people are increasingly concern about the health benefit of the food they eat. Nutrition quality covers a wide range of traits, for example, protein, amino acids, fat and phenolics. Naturally occurring variation for protein, amino acids, fat and fatty acid compositions have been under exploited. Only few genes have been identified and characterized. Formation of each nutrient in rice grain requires a complex pathway in which many genes or enzymes are involved. Current advances in protein and fatty acid biosynthesis in other crops and Arabidopsis may help to

understand the pathways in rice. Phenolics are expected to be an important field because they are proved to benefit human health in many ways (Shao and Bao, 2012). Genes for red pericarp formation, Rc (brown pericarp) on chromosome 7 and Rd (red pericarp) on chromosome 1 have been identified in rice germplasm but their roles in regulating the flavonoids biosynthesis are unknown. The genes for dark purple pericarp formation, *Pb* and *Pp*, are yet to be fine mapped and functionally characterized. MAS could be utilized to breed rice varieties accumulating anthocyanins (a characteristic of black rice) and proanthocyanidin (a characteristic of red rice). Genetic transformation could be conducted to breed rice with accumulation of the anthocyanins or proanthocyanidin in the endosperm, since these phytochemicals accumu late only in the bran layer (Shao and Bao, 2012).

5.3. Cooking and eating quality of brown rice

As concerns about nutritional quality rise, the consumption of brown rice will become more popular in near future. Cooking and eating quality of brown rice will be another issue. The knowledge that has been established for milled rice may not be applicable to the brown rice. It is needless to say that the genetic parameters controlling of the cooking and eating quality of brown rice, should be of priority consideration. Suitable cooking methods should be employed to make the brown rice more appealing to the consumers.

5.4. MAS with more genes/QTL

MAS with more genes/QTL together with suitable markers targeting many traits like Wx, *SSIIa*, and fragrance should be the important breeding objectives in a breeding program (Jin et al., 2010). MAS for quality and yield and resistance traits should be considered together in future. Strategies for more effective selection should be developed when many markers are used at the same time. In silica molecular breeding is coming into the era, with which alleles of different markers are designed in the computer; the phenotypes of new rice could also be designed and displayed in the computer.

6. CONCLUSION

Grain quality of rice as a whole is a complex trait that is comprised of appearance quality, milling quality, eating and cooking quality, and nutritional quality etc. Researches on the genetic control of the quality traits have made a great progress, especially for the appearance quality, cooking and eating quality. More genetic studies are needed for milling quality and nutritional quality. The progress on the molecular genetics on grain quality has allowed MAS to be used more efficiently. However, only MAS for cooking and eating quality and genetic engineering for nutritional quality have made some achievements. More molecular breeding

practices are needed for improvement of grain quality. With social development and improvement of living standards, cooking and eating quality of brown rice will be a new theme that deserves greater attention from researches. Studies including cooking methods, parameters for cooking and eating, genetics, and molecular breeding are among the top priorities.

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Genetic enhancement of seed quality traits in rice

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SUMMARY

High seed quality is important determinant for achieving higher rice production and also for food security. The target food grain production for the increasing population is a big challenge to meet under the adverse climatic effects. Majority of the seed quality traits are polygenic and complex in nature. Clear understandings on the inheritance of these traits are needed for improvement of these traits in rice breeding programs. Thus, QTL information and identification of robust markers for these traits are very important for improvement of the seed quality traits. The present review summarises the QTL identified, mapping population used and molecular markers detected for various seed quality traits such as seed vigour and its associated traits, seed dormancy, pre-harvest sprouting and seed storability in rice. Further, this chapter also focussed on the important seed quality traits and useful QTL for the rice breeders. Despite identification of several QTL, few have been fine mapped and limited targeted breeding efforts have been made in seed quality traits improvement programs. The gap in this area of research and the future research to be carried out for each trait are also discussed. The QTL that were already fine mapped and cloned for seed quality traits could successfully be utilised in marker assisted breeding for development of cultivar with improved seed quality traits.

Key words: Seed purity, QTL mapping, seed vigour, seed dormancy, seed longevity

1. INTRODUCTION

More than half of the global population consume rice as staple food for their calorie need and nutritional requirement (Gross et al., 2014). Increasing productivity of rice is a challenging task to feed the world population which is expected to increase by 33% in next thirty years, reaching 9.6 billion by 2050 (Manners and Van, 2018). Under the adverse effects of climate change causing high temperature, water crises, flood, drought and sea level rise along with surge in population growth are the challenges for the target production and productivity of rice across the globe. Seed is the basic and vital input for agriculture and distribution of the high-quality seed increases production. Quality seed acts like a mega factor for enhancing crop productivity (Chauhan et al., 2015). Seed quality is the degree of excellence in seed physical, physiological and genetic purity parameters that contribute to the performance of the seed (Hampton, 2002). Seed

physiological purity mainly determines the seed viability/seed storability, dormancy and seed vigour traits of seed. Improvement of seed quality attributes like seed vigour/seedling vigour and its associated traits such as seed dormancy, seed viability and seed longevity are essential for achieving high yield. In addition, good quality seeds also show resilience against adverse climate effects and biotic impediments to crop yields (Daniel, 2017). Traits mapped for various seed quality parameters are reported by many researchers in rice and those need to be deployed in breeding programs for enhancing seed quality parameters in rice varieties.

The most potential seed quality trait is the seed or seedling vigour that directly influences the crop productivity by delivering the genetic and yield potentials of the seed while ensuring uniformity in seed germination, seedling growth, establishment of seedling in the field and withstanding unfavourable environmental condition. This trait is important for direct seeded rice (DSR) system and this method of cultivation is very promising approach for cultivation of rice as it saves labour and energy (Aanandan et al., 2016). Improving the seed vigour of rice remains a primary breeding objective in rice (Finch-Savage and Bassel, 2016) as it is not only essential to enhance the yield but also ensures early uniform emergence which enhances crop establishment (Yamauchi and Winn, 1996). Better vigour plants can access the nutrients effectively and increase the plant's ability to compete against weeds (Zhao et al., 2006; Foolad et al., 2007; Egli et al., 2012) in direct seeding system of rice. Seed dormancy ensures a high rate of germination and control pre-harvest sprouting (PHS) in the field. Extremely strong dormancy leads to a low germination rate in the field and can even affect the final yield. Additionally, too weak dormancy leads to pre-harvest sprouting or vivipary especially in the high temperature and rainy environment during seed maturity thereby affecting the yield and sowing quality, storage quality of the seed and processing quality of the rice grain (He et al., 2014) resulting in significant financial loss to farmers and food processors. Hence, a moderate level of dormancy is required in rice crop to ensure a high rate of germination and also to control pre-harvest sprouting in the field. Seed longevity or seed viability after seed dry storage (storability) describes the total seed life span (Rajjou and Debeaujon, 2008). This storability period includes both the dormant and non-dormant states. During seed storage, seeds deteriorate, lose vigour, and, as a result, become more sensitive to stresses during germination, and ultimately die. The rate of this aging depends on the seed moisture content, temperature, and initial seed quality (Walters et al., 2005). Seed longevity is a key trait to secure seed viability and vigour of farmer's seed lots under the increased temperatures and humidity associated with climate change. It is a major determinant in rice seed production and germplasm

preservation. Seed longevity is a major challenge for the conservation of plant biodiversity and for crop success. This chapter describes the update information on the molecular mapping of various seed quality parameters in rice.

2. GENES REPORTED FOR CONTROLLING SEED/SEEDLING VIGOUR IN RICE

Genetics of seed/seedling vigour is complex in nature. The trait shows association with several physical, morphological, physiological and biochemical parameters that governs some metabolic events like seed germination and seedling growth. This trait is controlled by many quantitative trait loci (QTL) and quantitative inheritance of this trait has made it difficult to improve this genetic factor through conventional breeding. Hence, QTL analysis has been demonstrated as an effective tool to study the inheritance of the trait (Cui et al., 2002; Miura et al., 2002; Zhang et al., 2005a; Fujino et al., 2008; Wang et al., 2010; Dang et al., 2014; Liu et al., 2014; Zhang et al., 2017). Most of these detected QTL for rice seed/seedling vigour were found to be associated with various physical, physiological and biochemical traits related to seed such as seed weight, seed germination, germination rate, germination index, shoot length, root length, seedling length, seedling dry weight, radicle length, coleoptile length and mesocotyl length, amylase activity, reducing sugar, antioxidant traits and carotenoids are discussed. The information about QTL/gene associated with seed/seedling vigour will be helpful in marker-assisted breeding for improvement of seed/seedling vigour in rice.

The QTL controlling the trait, seedling vigour were reported to be associated with various seed quality traits (Table 1). Among these QTL, few major QTL were fine mapped and characterized. The QTL *qLTG-3-1*, a major QTL was fine mapped and cloned which controlled germination rate (GR) under various conditions. The QTL encodes a protein which may function to weaken the tissues that cover the embryo during germination (Fujino et al., 2008); another major QTL for seedling height, *qPHS3-2*, was fine-mapped and the candidate gene for seedling height was *OsGA20ox1* which controlled the gibberellins (GA) biosynthesis (Abe et al., 2012). Two major QTL, *qSV-1* and *qSV-5c* for root length were fine mapped and reported to influence seedling establishment and the germination rate (Xie et al., 2014). Another major QTL for seedling shoots length, *qSSL1b* was fine mapped by Zhang et al., 2017 which includes a candidate gene, *ph1* a major locus affecting plant height in rice which encoded a chitin-inducible gibberellins responsive protein (Kovi et al., 2011). Haplotype's analysis detected *OsML1* and *OsML2* through genome wide association mapping (GWAS) and were found to increase the mesocotyl length up to 4 cm (Zhao et al., 2018). Five candidate genes Os01g0166800, OS03g0799700 and Os03g0856700 (OsGA20ox1), Os04g0683600, and Os07g0600400 were inferred for seed vigour related traits *qSV1a, qSV3e,*

qSV4c, and *qSV7c* that were fine mapped (Chen et al., 2019). It was reported recently that disruption of an isopropylmalate synthase gene, *OsIPMS1*, resulted in low germination speed and seedling growth under various conditions due to its association with starch hydrolysis, glycolytic activity, and energy levels in germinating seeds (He et al., 2019). Gene controlling germination under stress condition was also identified by Kretzschmar et al., 2015 and it was reported that gene, *OsTPP7*, controls anaerobic germination by increasing the turnover of trehalose-6-phosphate (T6P) which enhances starch mobilization and driving the growth kinetics of the germinating embryo and the elongation of the coleoptile, which consequently enhanced anaerobic germination tolerance.

2.1 Mapping for physical and morphologic traits associated with seed/seedling vigour

2.1.1 Seed weight

A total of 17 QTL were detected for seed weight with phenotypic variance (PV) ranging from 2.2 to 15.9 % and located on chromosomes 1, 2, 3, 4, 5, 8, 9 and 11 (Cui et al., 2002; Zhang et al., 2017). Out of the 8 QTL identified by Zhang et al., 2017, three QTL were reported previously: *qTSW1* as *tgw1a* (Hua et al., 2002); *qTSW2 as qTGWT-2-2* (Zhuang et al., 2002) and *qTSW4b* was reported as *tgwt4* (Lin et al., 1996). The QTL, *qTSW1* was mapped to the locus overlapped with qSWW1a for seedling wet weight, consistent with significant correlation between 1000-seed weight and seedling wet weight (Zhang et al., 2017).

2.1.2 Shoot length (SL)/plant height (PH)/seedling height (SL)/stem length (SL) and stem diameter of seedling (SSD)

As many as 117 QTL controlling shoot length (SL) were reported and located on chromosome 1 to 12 and their PV ranged from 1.1 to 58.8 % (Redona and Mackill 1996; Huang et al., 2004; Zhang et al., 2005a; Zhou et al., 2006; Kanbar et al., 2006; Yang et al., 2010; Abe et al., 2012: Dang et al., 2014; Zhang et al., 2017; Singh et al., 2017; Yang et al., 2019; Chen et al., 2019; Yang et al., 2020). Eight QTL *viz.*, *qSSL1b*, *qSSL2b*, *qSSL3*, *qSSL4*, *qSSL5b*, *qSSL7*, *qSSL11b* and *qSSL12* reported by (Zhang et al., 2017 were close to those reported by Han et al., 2007, Cao et al., 2002, Abe et al., 2012, Dang et al., 2014 and Aanandan et al., 2016). The QTL, *qSHL3.1* and *qSHL5.1* identified by Singh et al., 2017 overlapped with earlier identified QTL for total seedling length (*qTL3-1* and *qTL5-1* of Kanbar et al., 2006). The QTL, *qSH-3* reported by Yang et al. (2020) also reported by Redona and Mackill (1996).

Two QTL for SSD (*qSD-2* and *qSSD 9*) were identified by Yang et al., 2019, 2020 which showed 6.86- 11.76 % PV. The QTL, *qSD-2* was same as *qSDW-2* identified by Han et al., 2007 and *qSSD 9* was reported as a stable QTL.

2.2 Mapping of physiological traits associated with seed vigour

The detected QTL for rice seed vigour were associated with many physiological traits *viz*., root length, shoot length, dry weight of seedling, germination rate, radicle length, root activity, coleoptile length, mesocotyl length, germination potential, germination index and time for 50 % germination (Regan et al., 1992; Redona and Mackill, 1996; Cui et al., 2002; Miura et al., 2002; Zhang et al., 2005a; Fujino et al., 2008; Wang et al., 2010; Dang et al., 2014; Xie et al., 2014; Liu et al., 2014; Zhang et al., 2017; Zhao et al., 2019; Sanghamitra et al., 2021). Some of the major QTL for physical and physiological traits associated with seed/seedling vigour are presented in Table 1.

2.2.1 Radicle length (RaL)

Two major QTL, *qSV-1* and *qSV-5c* present on the chromosome 1 and 5 were reported to regulate root length at low temperature and at normal temperature of 25^{0}C, respectively (Xie et al., 2014). In the QTL region, *qSV-5c* also reported to contain QTL for germination rate, reducing sugar content, root dry weight, total dry weight (Cui et al., 2002), and shoot length (Redona and Mackill1996). Whereas *qSV-1* regulate root length also found to influence the seedling establishment (Cui et al., 2002). A total of 7 novel QTL for radicle length (*qRL8a*, *qRL8b*, *qRL8c*, *qRL10a*, *qRL10b*, *qRL12a* and *qRL12b*) were detected under osmotic stress on chromosome 8,10 and 12 showing PV from 8.74-11.38% (Sabouri et al., 2013).

2.2.2 Coleoptile length (CL)

Twelve QTL controlling coleoptile length residing on chromosome 5, 8 and 11 with PV ranging from 12.7-28.8% were reported by Redona and Mc Kill 1996, Sabouri et al., 2013 and Xie et al., 2014. Out of these 12 QTL, *qSV-11* was reported as major QTL for coleoptile length which has an influence on germination (Xie et al., 2014).

2.2.3 Mesocotyl length (ML)

Five QTL for mesocotyl length were reported by Redona and Mackill, 1996. Later, 5 QTL were located for the trait on chromosome 1, 3, 7, 9 and 12 showing PV from 9.9 to 15.9% (Lee et al., 2012). However, two major QTL, *qMel-1* and *qMel-3* responsible for mesocotyl elongation present on chromosome 1 and 3 were fine mapped (Lee et al., 2012). Recent report of QTL and haplotypes affecting mesocotyl length were identified in a set of 621 rice accessions by GWAS, and the combinations of superior haplotypes of *OsML1* and *OsML2* reported to increase the mesocotyl length up to 4 cm (Zhao et al., 2018).

2.2.4 Germination rate (GR)

A total of 32 QTL controlling germination rate were identified on chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9 and 11, with PV ranging from 3.0 to 60.8% (Cui et al., 2002;

Zhang et al., 2005b; Wang et al., 2010; Diwan et al., 2013; Liu et al., 2014; Sabouri et al., 2013; Yang et al., 2019). The putative QTL, *qGR3-2*, *qTDW3-1* and *qSDW3-1*, shared the same interval of RG393-C1087 on chromosome 3. The QTL, *qGR5-1* for germination rate and *qRDW5-1* for root dry weight were both located in the marker interval of C246-RM26 on chromosome 5, while *qTDW5-1*, *qSDW5-1* and *qMRL5-1* shared the same interval of RM26-C1447. Similarly, another chromosomal region (R3166-RG360-C734b) on chromosome 5 was also identified to control germination rate, total dry weight, shoot dry weight and root dry weight (Cui et al., 2002).

The germination rate QTL, *qSV-8-1* identiûed by Zhang et al., 2005b was found to share the same map location with a major locus for osmotic adjustment and dehydration tolerance in rice, reported by (Lilley et al., 1996) and another germination rate QTL, *qSV-3-1* (Zhang et al., 2005b) was also reported by both Redona and Mackill (1996b) and Cui et al. (2002). The region of one major QTL *qGR-1* on chromosome 1 coincided with *gw1.1* and *gw1.2* for 1000-seed weight (Moncada et al., 2001) and the minor QTL *qGR-2* was on the similar location of *gw2.1* for 1000-seed weight (Marri et al., 2005) and grain width (Bai et al., 2010) on chromosome 2 (Wang et al., 2010). Out of the three major QTL identified by Liu et al., 2014, *qGR-8-2* was reported to be co-localized with *qSD40* for seed dormancy (Gu et al., 2004). Three minor QTL (*qGR-4-1*, *qGR4-2* and *qGR9*) reported as novel showed PV of 4.63-6.75 % were reported by Yang et al. (2019). A QTL, *qGR-9* was identified in the genomic region of *qGP-4*, which is related to the final germination percentage at 10 days (Wang et al., 2010). The gene for germination rate, *qLTG-3-1* was cloned which encoded a protein which function to weaken tissues that cover the embryo during germination (Fujino et al., 2008).

2.2.5 Germination index (GI)

Thirteen QTL associated with GI or speed of germination were found on chromosomes 1, 3, 7, 8, 9, 10 and 11 with phenotypic variance which ranged from 3.85 to 54.9% (Wang et al., 2010; Liu et al., 2014; Yang et al., 2019). A major QTL, *qGI-8* was located (Liu et al., 2014) in the same region of *qSD8* for seed dormancy (Gu et al., 2004). Out of the 13 QTL, two QTL (*qGI-9-1*and *qGI 9-2*) with PV of 3.85-8.22% were novel (Yang et al., 2019). Disruption of an isopropylmalate synthase gene, *OsIPMS1* resulted in low germination speed and seedling growth under various conditions (He et al., 2019). It regulates seed vigour by its association with starch hydrolysis, glycolytic activity, and energy levels in germinating seeds.

2.2.6 Seed vitality Index (SVI)

Seed vitality index is a measure of seed germination index and seedling length. Yang et al. (2019) detected four QTL (*qVI-1*, *qVI-7*, *qVI-9-1* and *qVI-9-2*) with PV

ranging from 3.60 -5.88 %. The QTL *qVI-1* was detected to be co-located with known QTL, *qGR-1* for germination rate reported by Wang et al. (2010).

2.2.7 Germination percentage (G% / GP% / FGR%)

A total of 41 QTL found to influence germination percentage were present on chromosomes 1, 3, 4, 5, 6, 7, 8, 9, 10, 11 and 12. The phenotypic variance explained ranged from 4.42 to 40.67 (Wang et al., 2010; Sabouri et al., 2013; Liu et al., 2014; Xie et al., 2014; Yang et al., 2019; Najeeb et al., 2020). The QTL, *qGP-4-1* reported by Yang et al., 2019 was identified in the genomic region of *qGP-4* for germination percentage at 10 days (Wang et al., 2010). As per reports of Wang et al. (2010), a major *qGP-6* was detected and *qGP-11* is near to the 1000-seed weight QTL, *qgw11* (Li et al., 2000). Furthermore, *qGP-4* located on the same region of *qSD4* for seed dormancy on chromosome 4 (Gu et al., 2004). Co-localized additive QTL *qGP3*, *qGI3* and *qT503.2* in the marker interval of RM130–RM3684 on chromosome 3 and *qGP7*, *qGR7*, *qGI7* and *qT507.1* in the interval of RM8261–RM5426 on the chromosome 7 were detected. These co-localized QTL could be very useful in the simultaneous improvement of more than one trait. Sabouri et al., 2013 also identified *qGP-8* as major QTL for germination percentage under osmotic stress which was collocated with cold tolerance gene *Os01g74470* (Shakiba et al., 2017). Recently, a QTL *qGP-9* reported a novel gene responsible for GP by Yang et al., 2019 and also *qLTG(I)* was reported by Najeeb et al. (2020) as major QTL for germination at low temperature (12-14^{0}C). Of all these QTL reported, only major QTL, *qSV-5c* present on chromosome 5 within marker interval of RM26–Xbin818 with PV of 28.8% was fine mapped and found responsible for seed germination at optimum and at low temperature (15^{0}C) (Xie et al., 2014).

Genes/QTL controlling seed germination under stress conditions were also reported. Gene, *OsTPP7* was reported to control anaerobic germination by increasing the turnover of trehalose-6-phosphate (T6P), thus enhancing starch mobilization and driving the growth kinetics of the germinating embryo and the elongation of the coleoptile, which consequently enhanced anaerobic germination tolerance (Kretzschmar et al., 2015). Further, *OsIPMS1* also reported to be involved in starch hydrolysis, glycolytic activity and energy levels in germinating seeds which influenced seed vigour (He et al., 2019). Map based cloning results of major effective QTL, *qLTG 3 1* for seed germination at low temperature encoded an unknown protein that was specifically expressed in the embryo, panicle, and shoot influenced the germination by accelerating vacuolation that might be involved in weakening of the tissues covering the embryo (Fujino et al., 2008). However, Fujino and Matsuda, 2010 showed that *qLTG31* unregulated the genes involved in phytoalexin biosynthesis and increased the expression of probenazole induced protein (PBZ1) which might have induced programmed cell death.

2.2.8 Time for 50% germination (T50%)

Seven QTL associated with T50% were detected and located on the chromosomes 1, 3 and 7 at three different stages of harvesting (4,5 and 6 weeks after heading) with phenotypic variance which varied from 8.88 to 16.89 (Liu et al., 2014). Among these QTL, *qT501.2* was found to be co-located with *qGI1.2* for germination index between the marker interval RM6950 and RM5759 and with *qGRL1.1* for grain length, *qSD1* for seed dormancy (Wang et al., 2012). In addition, *qT501.1* on chromosome 1 also coincided with *qGL-1* for grain length (Wan et al., 2005). The QTL, *qT503.1* was in the same position with QTL for seed dormancy, *qSD3.2* on the chromosome 3 (Cheng et al., 2014). In addition, *qT507.1* was near to *Sdr4* which controlled seed dormancy (Sugimoto et al., 2010) and *qGL7-2* for grain length in rice (Shao et al., 2010). The QTL on chromosome 7, *qT507.2* was at same location with QTL, *qGL7* for grain length (Bai et al., 2010).

2.2.9 Initial growth rate (IGR), early uniform emergence (EUE), early vigour (EV) and seedling establishment

Two major QTL for early-stage plant development (*qEPD1* and *qEPD2*) that control initial growth rate of seedling were mapped. It was predicted that *qEPD1* and *qEPD2* correspond to gibberellin 20 oxidase-1 and gibberellin 20 oxidase-2 genes (*OsGA20ox1,2*). The major QTL, *qEPD2* controls plant height at initial growth stages is present on chromosome number 3 where as *qEPD1* on chromosome 1 modulates plant growth both at initial and heading stages (Yano et al., 2012). Six QTL for early uniform emergence were detected by Singh et al., 2017. Two QTL on chromosome 3 (*qEUE3.1* and *qEUE3.2*), one on chromosome 4 (*qEUE4.1*), two on chromosome 5 (*qEUE5.1* and *qEUE5.2*), and one on chromosome 6 (*qEUE6.1*) were reported by the group. Many QTL for traits such as seed dormancy (Miura et al., 2002), germination rate, shoot/RDW, and for physiological traits namely reducing sugar and amylase activity were mapped in a region similar to *qEUE5.1* (Cui et al., 2002; Zhang et al., 2005a). The team also reported 17 QTL for EV and located on chromosome 3, 4, 5 and 6 exhibiting PV from 5.8 to 13.1 % (Singh et al., 2017). The QTL *qEV3.1*, *qEV3.2*, and *qEV5.1* coincided well with QTL identified by earlier researchers (Zhang et al., 2005a; Lu et al., 2007; Zhou et al., 2006; Xie et al., 2014). The QTL, *qDTY3.1* reported for grain yield under drought (Dixit et al., 2014), overlapped with *qEV3.2*. This indicates that these physiological components might have a direct relationship with seed vigour and may possibly supply energy for fast and uniform seedling growth. As per the findings of Yang et al., 2020, four candidate genes viz., protein gene (*Os01g0655500*), receptor-like protein kinase gene (*Os03g0643250*), flavin-containing monooxygenase family protein gene (Os09g0549300) and HMGd1 protein gene (Os09g0551600) mainly control early seedling growth/vigour in rice. Whereas disruption of an isopropylmalate

synthase gene, *OsIPMS1* reduced the germination speed and seedling growth of rice (He et al., 2019). A major QTL, *qSV1* for seedling establishment at low temperature was fine mapped and located within marker interval of G393–C86 with PV of 22.6% (Xie et al., 2014).

2.2.10 Shoot and root traits

2.2.10.1 Shoot weight (SFW/SDW)

Eleven QTL for shoot fresh weight (SFW) were detected on chromosome 1, 2, 3, 5, 6, 7, 8, 9, 10 (Singh et al., 2017; Yang et al., 2019; Yang et al., 2020). The QTL, $qSFW_{3.1}$ for SFW was detected at both 8 days after sowing (DAS) and 21 DAS while, $qSFW_{5.2}$ was detected only at 8 DAS indicated the influence of SFW at the initial growth stage of the seedlings (Singh et al., 2017). Among these QTL, *qSFW-8* was reported as a major QTL (Yang et al., 2019) while *qSFW-9* was identified as a consistent QTL (Yang et al., 2020). A total of 16 QTL controlling shoot dry weight (SDW) were detected on the regions of chromosome 1, 3, 5, 6, 9 and 12 showing PV ranging from 3.0 to 26.9 % (Cui et al., 2002; Dang et al., 2014; Singh et al., 2017). The QTL for shoot weight (*qSDW5.1*) detected at 8 DAS on chromosome 5, explained phenotypic variance of 7.9% during initial seedling growth stages (Singh et al., 2017). Nineteen QTL influencing seedling wet weight (SWW) were detected on the chromosomes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 and 12 (Zhang et al., 2017; Singh et al., 2017; Yang et al., 2020). Most of these QTL reported to share the same locus with SDW.

2.2.10.2 Seedling dry weight (SDW)/Total dry weight (TDW)

As many as 77 QTL/markers related to total seedling dry weight were reported from various regions on chromosome1, 2,3, 4, 5, 6, 8, 9, 10 and 12 using different mapping populations by different researchers (Cui et al., 2002; Zhang et al., 2005b, 2017; Zhou et al., 2006; Kanbar et al., 2006; Yang et al., 2010; Diwan et al., 2013; Cheng et al., 2013; Singh et al., 2017; Chen et al., 2019). The QTL for seedling dry weight on chromosome 6, named as *qSDW6b* was reported to share the same locus with *qTDW6-2* reported by Cui et al. (2002). The locus *qSDW1c* was also reported to contain the major QTL *qSSL1b* responsible for shoot length which was fine mapped by Zhang et al., 2017. The QTL, $qTDW_{5.1}$ reported by Singh et al. (2017) was reported earlier by Cui et al. (2002) and Diwan et al. (2013). Out of seven QTL (*qSV2a*, *qSV4b*, *qSV5c*, *qSV6b*, *qSV7c*, *qSV8d*, and *qSV11a*) reported by Chen et al. (2019), two were previously reported (*qSV2a* for mesocotyl length by Zhao et al. (2018)) and *qRL-2* for root length by Cairns et al. (2009)); *qSV4b* was mapped on the same position with *qSDW4.2* for shoot dry weight (Cheng et al., 2013) and *qGP-4* for germination percentage (Wang et al., 2010); *qSV5c* co-localized with *qFV-5-2* for seedling height (Zhou et al., 2007) and *qSEV-5-2* with both PH and plant dry weight (Lu et al., 2007), *qSV6b* was close to *qDW-6* for seedling dry

weight (Wang et al., 2010) and *qLDW-6* for leaf biomass (Cairns et al., 2009); *qSV7c* was mapped with *qSDW5-1* for shoot dry weight (Cui et al., 2002) and *qSL-7* for shoot length (Cairns et al., 2009); *qSV8d* was mapped with *qSV-8-2* for germination rate, shoot length, and root length (Zhang et al., 2005b) and with *qPR-8* for biomass portioning to roots (Cairns et al., 2009), while *qSV11a* was mapped with *qFML11-1*for mesocotyl length (Zhao et al., 2018) and with qLN-11 for leaf number (Cairns et al., 2009). Of all these QTL, only QTL *qSV7c* was fine mapped and the candidate gene was Os07g0600400 found control seedling dry weight, plant height and tiller number (Chen et al., 2019).

2.2.10.3 Root activity

Root activity is a measure of oxidation (±-naphthylamine) ability of fresh root. Three QTL were reported for root activity and located on chromosome 5 and 9 with PV ranging from 4.6-13.0 %. QTL for root activity and seedling dry weight were reported to have similar locations on chromosome 5 with opposite effects (Cui et al., 2002). The QTL, *qRA5-2* was located between R3166 and R830, where two QTL for total amylase activity and reducing-sugar content traits were also detected (Cui et al., 2002).

2.2.10.4 Root length (RL)

Thirty-seven QTL controlling root length were located on the chromosomes of 1, 2, 3, 4-1, 4-2, 5, 6, 7, 8, 9, 10 and 12, and PV ranged from 3.07 to 27.1 % (Redona and Mackill, 1996; Cui et al., 2002; Zhang et al., 2005a; Yang et al., 2010; Dang et al., 2014; Zhang et al., 2017; Yang et al., 2019; Yang et al., 2020). The QTL, *qRL-1* mapped by high density genome mapping of RIL population detected stable QTL and *qRL-4* was also identified by Courtois et al., 2003; Yang et al., 2020).

2.2.10.5 Root fresh weight (RFW), root dry weight (RDW) and root diameter (RD)

Three QTL controlling root fresh weight were located on the chromosomes of 2, 3 and 9 and the proportion of phenotypic variance explained (PV) from 8.27 to 10.98 % (Yang et al., 2019; Yang et al., 2020). Among these three QTL, *qRFW-3* and *qRFW-9* were found stable QTL (Yang et al., 2020). Five QTL (*qRDW1*, *qRDW5-1*, *qRDW5-2*, *qRDW10* and, *qRDW3.1*) for root dry weight were detected and located on chromosome 1, 3, 5 and 10 with PV ranging from 2.9 to 24.1% (Cui et al., 2002; Singh et al., 2017). Of these five QTL, *qRDW5-1* was reported as major QTL (Cui et al., 2002). Four QTL (*qRD-2*, *qRD-4*, *qRD-8*, *qRD-9*) controlling root diameter were detected which showed PV from 4.85-6.83% and located on chromosome 2, 4, 8 and 9 (Yang et al., 2019; Yang et al., 2020).

2.2.10.6 Root volume (RV), root surface area (RSA), root thickness (RT)

Five QTL (*qRV-2*, *qRV-3*, *qRV-8*, *qRV-9* and *qRV-11*) showing PV from 4.14 - 10.47% were identified which controlled the root volume (Yang et al., 2019; Yang et al., 2020). Of these five QTL, *qRV-9* was also identified by Ikeda et al., 2006 and *qRV-3* was found stable QTL (Yang et al., 2020). Root surface area was found to be controlled by four QTL (*qRSA-2*, *qRSA-3*, *qRSA-9* and *qRSA-11*) present on the chromosome 2, 3, 9 and 11 with PV varying from 5.69 -9.07 % were reported (Yang et al., 2019; Yang et al., 2020). Of these four QTL, *qRSA-3* reported by Yang et al. (2020) was found stable QTL which was also identified by Zhang et al., 2017. Root thickness was reported to be controlled by 21 QTL which showed PV from 4.64 to 21.14% (Zhao et al., 2019). Out of these QTL, 10 were already reported by Courtois et al. (2009).

2.3 Biochemical traits associated with seed/seedling vigour

Few QTL for biochemical traits are reported to be associated with seed/seedling vigour. Cui et al. (2002) detected QTL related to biochemical traits such as amylase activity and reducing sugar associated with seed vigour in rice. Three QTL were detected for total amylase activity located on chromosome 3, 5 and 6 with PV ranging from 4-11%. The QTL for total amylase activity, *qTAA3-1* on chromosome 3 closely linked to QTL for germination rate, *qGR3-3*. Two QTL, *qTAA6-1* for TAA and *qAAA6-2* for AAA, were located within the interval of Waxy-C1496. Six QTL were identified for reducing-sugar content and located on chromosome 3, 5, 6, 10 and 11 showing PV from 2.9-18.4%. Out of these 6 QTL, one QTL, *qRS3-1*, was located within the locus for germination rate, total dry weight, and shoot dry weight. Another QTL, *qRS5-1* located near *qTAA5-1* for total amylase activity on chromosome 5. Interestingly, *qRS6-2* was located within the same interval of Waxy-C1496 as that of *qAAA6-2* for ±-amylase activity, *qTAA6-1* for total amylase activity along with four QTL of seedling-vigour traits. The largest effect QTL for root activity (RA) was found to be *qRA5-1*, was detected within C246-RM26, where several QTL for seedling-vigour traits were identified. The QTL, *qRA5-2* was located in between R3166 and R830, where two QTL for total amylase activity and reducing-sugar content traits were also detected. This co-localization or close linkage of several QTL provides a genetic basis underlying the correlation among the traits.

In an association study of antioxidant traits and enzymes including chlorophyll and carotenoid content related to seed vigour with markers showed marker-trait association for chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, starch, amylase, total anthocyanin content, gamma-oryzanols, total phenolics compounds, total flavonoids, catalase, and super oxide dismutase (Sahoo et al., 2020). Apart from these biochemical traits, many other biochemical traits

such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), nitrate reductase activity, glutamic acid decarboxylase activity, salicylic acid and seed protein content reported to be associated with seed vigour (Mahendra et al., 2015; Huang et al., 2017). Further, QTL validation and utilization in rice improvement programs are needed. Though few genes necessary for antioxidant traits in rice have been reported and fine mapped also, more gene/ loci need to be identified to explain the complexities of these traits (Furukawa et al., 2006; Kim et al., 2007; Xu et al., 2017).

3. IMPROVEMENT FOR PRE-HARVEST SPROUTING (PHS) AND SEED DORMANCY (SD)

Seed dormancy is a complex trait controlled by many genes that are quantitatively inherited and are influenced by environmental factors (Takahashi, 1980; Basbouss-Serhal et al., 2016). The primary dormancy is developed on the mother plant before seed maturation which coincides with the accumulation of storage compounds, the acquisition of desiccation tolerance, the quiescence of metabolic activity, increased levels of the plant hormone abscisic acid (ABA) (Holdsworth et al., 2008). Breakage of seed dormancy causes pre-harvest sprouting. Genetic variation for seed dormancy exists abundantly in rice. Further, seed dormancy is also reported to be associated with red pericarp colour of the grain enriched with flavonoids. Also, majority of *japonica* rice varieties are weaker in seed dormancy than *indica* (Lee et al., 2016). In addition, *japonica* rice with awn has higher PHS resistance compared to *indica* varieties (Sun et al., 2015; Lee et al., 2017). Hence, understanding of the genetic bases of SD and its breakage is a major breeding target in rice to solve the problem with PHS (Zhang et al., 2014).

3.1 QTL mapped for SD and PHS

The QTL controlling pre-harvest sprouting and seed dormancy are presented in the Table 2 Out of the 160 QTL reported for SD and PHS, few QTL are also fine mapped and cloned. The first cloned gene, *Sdr4* positively regulated by *OsVP1* and influenced seed dormancy (Sugimoto et al., 2010). The locus, *qSD12* includes two candidate genes namely *PIL5* (phytochrome interacting factor3-like5) and *bHLH* (basic helix-loop-helix) (Gu et al., 2010). The pleiotropic gene, *qSD7-1/qPC7* (Rc locus) promotes the expression of key genes for ABA biosynthesis and seed pigmentation (Gu et al., 2011). Seed dormancy associated with *qSD1-2* on chromosome 1, was reported to be within a 20-kb region containing the candidate gene, *OsGA20ox2* (Ye et al., 2015). The QTL, *qSD3.1* and *qSD3.2* were proposed to control dormancy through ABA path way (Zhang et al., 2020). Recently Yuan et al. (2020) had reported three new QTL, *qDOM3.1*, *qDOM6.2*, and *qDOM10.2* for seed dormancy. The candidate gene *LOC_Os03g01540* for *qDOM3.1* was involved in the ABA signalling pathway. Gibberellin (GA) regulating the

development of endosperm-imposed dormancy, *qSD1-2* reported in weedy rice (Ye et al., 2015). The candidate gene for gibberellins (GA) synthesis was reported as *OsGA20ox2*.

Several QTL for SD and PHS were also found collocated with other traits such as seed pericarp colour and heading date. Weedy red rice QTL, *qSD7-1* that influence dormancy by controlling ABA synthesis also influenced red pericarp color as it was tightly linked with Rc, the red pericarp colour gene (Gu et al., 2011). It was further reported that the same transcription factor gene (*Os07g1120*) regulate both abscisic acid (a dormancy-inducing hormone) and flavonoid (red pigments) biosynthesis pathways (Gu et al., 2011) which explain the stronger SD in black-hulled awned red rice population (Delouche et al., 2007). Collocation was also reported for the SD/heading date QTL on Chromosome 3 (Sdr1/Hd8; Takeuchi et al., 2003) and 6 (*qSD-6-2/qHD-6*; Jing et al., 2008), with the dormancy-enhancing alleles delaying flowering. QTL reported to have specifically PHS tolerance were *qSD-1*, *Sdr6*, *qDEG1*, *qSD1.1*, *qSnd-1*, *qPHS1*, *qSDR9.1*, *qSDR9.2*, *qPHS3*, *qPHS4*, *qPHS 11* (Miura et al., 2002; Gu et al., 2006; Li et al., 2011; Lu et al., 2011; Marzougui et al., 2012; Wang et al., 2014; Jang et al., 2020; Mizuno et al., 2018; Xu et al., 2019; Cheon et al., 2020). Some of the stable major QTL such as *qPHS1* (Jang et al., 2020), *qSDR9.1* and *qSDR 9.2* (Mizuno et al., 2018) and *qPHS3*, *qPHS4*, *qPHS-11* (Cheon et al., 2020) conferred PHS resistance under variable environment and will be useful in PHS improvement programs in rice.

It is observed that close relationship exist between resistance to PHS and low temperature germination (Hori et al., 2010; Jang et al., 2020). The major QTL identified for PHS resistance on the short arm of chromosome 3 was localized to a 474-kbp region within marker interval of RM14240 and RM14275 was also flanked the low-temperature germinability gene, *qLTG3-1* (Fujino et al., 2008). The QTL, *qLTG3-1* causes tissue weakening of epiblast covering the coleoptile and aleurone layers which resulted in reduced mechanical resistance to the growth potential of the coleoptile. The region of *qSDR9.2* overlapped with the region of Delayed Seed Germination 1 (*OsDSG1*) in *Oryza sativa* (Mizuno et al., 2018) (Park et al., 2010). S1 loci reported in *indica* rice, was a variant in the hypothetical protein gene (OS01G0162900) included in the chromosomal region of germination related QTL Sdr6 (Marzougui et al., 2012), *qSD-1* (Miura et al., 2002), *qSD1* (Gu et al., 2004, 2006), and *qDEG1* (Li et al., 2011). Genes containing these significant SNP loci and overlapping with reported QTL regions that represent new alleles in natural populations might be functional genes for the regulation of PHS (Lee et al., 2017). The QTL that were cloned and those were fine mapped facilitated understanding the genetic architecture for seed dormancy and germination in rice. These QTL could further be used for marker assisted breeding programs for improvement of PHS resistance.

4. IMPROVEMENT FOR SEED LONGEVITY/SEED STORABILITY

Seeds possess a wide range of systems (protection, detoxification and repair) allowing them to survive in the dry state and to preserve a high germination ability. Many characters inherited such as, seed size, harvesting time, dormancy, and storage conditions, are reported to be related to storability (Lin et al., 2015). Seed longevity not only depends on storage conditions but also on genetic character of seed. In rice seed, it has also been noted that there is a strong correlation between seed longevity and desiccation tolerance (Ellis and Hong, 1994). During the storage of rice seed alpha-amylase activity declines and initiation of gelatinization is more intense in *indica* variety than the *japonica*, which suggests that *indica* types show more seed longevity compared to *japonica* (Dong et al., 2017). Duration of seed longevity was reported to vary between species and also vary within a species (Lee et al., 2007; Wang et al., 2018). In case of rice, *aus* and *indica* varieties possess greater seed longevity in comparison with other sub-groups, especially temperate *japonica*, has relatively short seed longevity (Kameswara et al., 1996; Hay et al., 2015). Seed longevity is a quantitative trait that varies in degree and can be attributed to polygenic effects that is governed by two or more genes and their environment. The causes behind so much variation in seed longevity is still unclear. QTL mapping is the most employed breeding approach for genetic analysis of this trait so as to identify markers for seed longevity for its utilisation in marker assisted breeding

4.1 QTL for seed longevity

Out of a total of 130 QTL reported for seed longevity, the major QTL controlling the trait are listed in the Table 3. QTL, *qLG-9/qSS-9*, *qSS1* and *qSS3.1* are fine mapped (Sasaki et al., 2015; Lin et al., 2015; Yuan et al., 2019). Fine mapping of the QTL, *qLG9* identified trehalose-6-phosphate phosphatise (TPP: *Os09g0369400*) and an unknown protein (*Os09g0369500*) encoding region (Sasaki et al., 2015). TPPs catalyse the dephosphorylation of trehalose-6-phosphate to trehalose known to be involved in anaerobic germination tolerance (Iordachescu and Imai, 2008; Kretzschmar et al., 2015). Fine mapping of *qSS9* (Lin et al., 2015) identified 12 candidate genes in the interval between markers Y10 and Y13. Out of these, one gene is *Os09g0368900*, a DUF632 domain containing protein of unknown function. Two genes, *Os09g0368200* and *Os09g0368500*, were similar to a polyamine oxidase (PAO) precursor involved in plant growth and development. *Os09g0369050* was similar to dehydration-responsive element (DRE) binding factor 2. The DRE is a cis-acting element including a core sequence of A/GCCGAC and is involved in both cold-and dehydration-inducible gene expression (Yamaguchi-Shinozaki and Shinozaki, 1994).

Table 1. Major QTL/marker for seed/seedling vigour related to physical and physiological traits from different population of rice

Sl. No.	Traits	QTL	Marker interval/ nearest marker/ position(cM)	Population used	Chr.	LOD	References	Marker	Known QTL/ colocalized QTL/QTL reported in the previous studies
1.	Seed weight	*qSW5-1*	R3166-RG360	RILs (Zhenshan 97/ Minghui63)	5	23.4	Cui et al. (2002a)	RFLP	
		qTSW2	134.00–148.00	RILs (93-11/ PA64s)	2	3.61	Zhang et al. (2017)	SNP	*qTGWT-2-2*, Zhuang et al. (2002)
2.	Radicle length	*qSV-1*	G393–C86	RILs (ZS97/MH63)	1	11.86	Xie et al. (2014)	SSR/RFLP	
		qSV-5c	RM26–Xbin818	RILs (ZS97/MH63)	5	14.8	Xie et al. (2014)	SSR/RFLP	
3.	Coleoptile length	*qSV 11*	Xbin1477–Xbin1505	RILs (ZS97/MH63	11	13.02	Xie et al. (2014)	SSR/RFLP	
4.	Mesocotyl length	*qMel-1*	RM5448–RM3482	BILs (Kasalath/ Nipponbar)	1	5.4	Lee et al. (2012)	SSR	
		qMel-3	RM8277–RM1221	BILs (Kasalath/ Nipponbar)	3	7.5	Lee et al. (2012)	SSR	
5.	Germination rate	*qGR5-1*	C246-RM26	RILs (Zhenshan97/ Minghui63)	5	15.9	Cuiet al. (2002a)	RFLP	
		qLTG 3 1	GBR3001*-GBR3002	BILs (Hayamasari/ Italica Livorno)	3	15.7	Fujino et al. (2004)	RFLP/SSR	
		qGR-1	RM9–RM7075	RILs (Daguandao/ IR28)	1	11.0	Wang et al. (2010)	SSR	*gw1.1 & gw 1.2* for 1000-seed weight, Moncada et al. (2001)

Sl. No.	Traits	QTL	Marker interval/ nearest marker/ position(cM)	Population used	Chr.	LOD	References	Marker	Known QTL/ colocalized QTL/QTL reported in the previous studies
		qGR8.2	*RM6976-RM6845*	RIL (Jiucaiqing/IR26)	*8*	*6.74*	Liu et al. (2014)	SSR	*qSD8*, Gu et al. (2005)
		qGR12	*RM1880-RM20*		*12*	*2.73*			
		qGR2	RM341–RM106	DH (IR64/Azucena)	2	4.5	Diwan et al. (2013)	SSR	gw2.1 for 1000-seed weight (Marri et al., 2005) and grain width (Bai et al., 2010)
		qSV-3-2	RM148–RM85	RILs (Lemont/Teqing)	3	5.4	Zhang et al. (2005b)	RFLP/ microsatellite	
6.	Germination index:	*qGI-11*	RM3428–RM6091	RILs (Daguandao /IR28)	11	5.1	Wang et al. (2010)	SSR	
		qGI-8	RM6976-RM6845	RILs (Jiucaiqing/ IR26)	8	4.95	Liu et al. (2014)	SSR	*qSD8*, Gu et al. (2005)
7.	Germination %/Germination potential %	*qGP-6*	RM528-RM340	RILs (Daguandao /IR28)	6	5.3	Wang et al. (2010)	SSR	
		qSV-5c	RM26–Xbin818	RILs (ZS97/MH63)	5	14.8	Xie et al. (2014)	SSR/RFLP	
		qSV-11	Xbin1477–Xbin1505 1	RILs (ZS97/MH63)	11	13.02	Xie et al. (2014)	SSR/RFLP	
		qGP-8	RM6208- RM8264	F2-F3 (Tarommahalli/ Khazar)	8	5.19	Sabouri et al. (2013)	SSR	
		$qLTG(l)_1$	75SNP_1_42425574	BILs (Weed Tolerant Rice-1/Haoannong)	1	7.31	Najeeb et al. (2020)	SNP	
8	Initial growth rate:	*qEPD-1*	A-1255Ga (138.2)–A-2056Gt (155.2)	BILs (Koshihikari/ Habataki)	1	4	Yano et al. (2012)	SNP	

Sl. No.	Traits	QTL	Marker interval/ nearest marker/ position(cM)	Population used	Chr.	LOD	References	Marker	Known QTL/ colocalized QTL/QTL reported in the previous studies
		qEPD-2	A-1369Tc (158.5)–A-362Tc (180.5)		3	5.2			
9.	Seedling establishment	*qSV-1*	G393–C86	RILs (ZS97/MH63)	1	22.6	Xie et al. (2014)	SSR/RFLP	
10	Shoot length	*qFV-5-2*	RM161–CDSR49	RILs (Lemont/Teqing)	5	8.76	Zhou et al. (2007)	RFLP/ microsatellite	
		qSH-3	RM545–RM3766	RILs (Xiushui 79/C-Bao)	3	6.13	Yang et al. (2010)	SSR	
		qSV-3-1	RM16–RM168	RILs (Lemont/Teqing)	3	5.7	Zhang et al. (2005b)	RFLP/ microsatellite	
		qSL-2	RM324–RM166	BC3F3 (Vandana/ Moroberekan)	2	4.13	Cairns et al. (2009)		
11	Plant height	*qpht3-1*	RZ313	DH (CT9993/IR62266)	3	7.87	Kanbar et al. (2006)	RFLP/AFLP/ microsatellite	
		qpht3-2	RG369		3	7.12			
		qpht 10-1	G333		10	5.04			
		qSV3e		Natural population	3		Chen et al. (2019)	SNP	
		qSV4c			4				
		qSV 7c			7				
12	Seedling height	*qCSH2*	RM262–4RM263	F_2–F_3 (Milyang23/ Jileng1)	2	5.2	Han et al. (2007)		
		qSSL1 b	117.12–133.43	RILs (Lingshui/ Hangzhou)	1	5.48	Zhang et al. (2017)	SNP	
		qPHS3-2	RM1373–RM7389	RILs (Kakehashi/ DunghanShali)	3	17.5	Abe et al. (2012)	SSR	
		qPHS1	RM8085–RM1339		1	5.5			
13	Stemlength	*qsl2-1*	EMP2_8	DH (CT9993/IR62266)	2	10.24	Kanbar et al. (2006)	RFLP/AFLP/ microsatellite	
		qsl2-2	*RG158*		2	7.32			

Sl. No.	Traits	QTL	Marker interval/ nearest marker/ position(cM)	Population used	Chr.	LOD	References	Marker	Known QTL/ colocalized QTL/QTL reported in the previous studies
14	Vigour index /Vitality index	*qVI*	RM162	DH (IR64/Azucena)	5	4.4	Diwan et al. (2013)	SSR	
15	Shoot fresh weight	*qSFW-8*	Block14052-Block14058	RILs (H335/CHA1)	8	9.18	Yang et al. (2019)	SNP	
16	Shoot dry weight	*RM 276* *RM 314,* *RM528*	33.5 33.6 100.8	Natural population	- - -	6	Dang et al. (2014)	SSR	
17	Seedling fresh weight (SFW)	*qtfw7-1*	RG404	DH (CT9993/IR62266)	7	4.00	Kanbar et al. (2006)	RFLP/AFLP/ microsatellite	
		qSFW-8	Block14052-Block14058	RILs (H335/CHA1)	8	9.18	Yang et al. (2019)	SNP	
18	Seedling dry weight(SDW)	*qFV-5-1*	RM13–N10150 –Y1049	RILs (Lemont/Teqing)	5	5.58	Zhou et al. (2007)	RFLP/ microsatellite	
		qSDW5-1	RM26–C1447	RILs (Zhenshan97/ Minghui63)	7	7.91	Cui et al. (2002a)	RFLP	
		qDW5	RM87–RM334	DH (IR64/Azucena)	5	2.9	Cui et al. (2002b)	RFLP	Zhang et al. (2005a), Diwan et al. (2013)
		qSDW4.2 *qSDW8.2*	RM273–RM3839 RM342–RM3459	RILs (Jiucaiqing/IR26)	4 8	7.06 4.43	Cheng 'et al. (2013)	SSR	
		qSDW2	ME4_16	F2–F3 (Milyang 23/ Jileng 1)	12	5.35	Han et al. (2007)		
		qtdw12-1	*ME4_16*	DH (CT9993/IR62266)	12	5.35	Kanbar et al. (2006)	RFLP/AFLP/ microsatellite	
		qSV7c		Natural population	7		Chen et al. (2019)	SNP	

Sl. No.	Traits	QTL	Marker interval/ nearest marker/ position(cM)	Population used	Chr.	LOD	References	Marker	Known QTL/ colocalized QTL/QTL reported in the previous studies
19	Root length (RL)	*qMRL6-1*	C952-waxy	RILs (Zhenshan 97/ Minghui63)	6	12.4	Cui et al. (2002a)	RFLP	
		qSV-8-2	RM230–RM264	RILs (Lemont/Teqing)	8	5.3	Zhang et al. (2005b)	RFLP/ microsatellite	
		qRL-1	RM486–RM26	RILs (Xiushui79/ C-Bao)	1	5.0	Yanget al. (2010)	SSR	
		qRL-2	RM2127–1RM48		2	3.6			
		RM3453	25.4	Natural population	1		Dang et al. (2014)	SSR	
		RM5389	124.4		1				
20	Root dry	*qRDW5-1*	C246–RM26	RILs (Zhenshan97/ Minghui63)	5	19.4	Cuiet al. (2002a)	RFLP	
21	Leaf area	*qlA-1*	RM297	BC_3F_3 (Vandana/ Moroberekan)	1	5.23	Cairnset al. (2009)		
22.	Root to shoot fresh weight ratio	qrsfwr 7-1	EM11-1	DH (CT9993/IR62266)	7	4.79	Kanbar et al(2006)	RFLP/AFLP/ microsatellite	
		qrsfwr 7-2	RG404		7	6.26			

QTL associated with seed longevity collocated with traits seedling dry weight and total dry weight (Sasaki et al., 2005). The QTL (*qAOC3.1*) for antioxidant capability colocalized with *qSS3.1* for seed longevity on chromosome 3 (Yuan et al., 2019). Another QTL, *qSS3.1* also co-localized with candidate gene (*OsFAH2*) for seed storability. Recently, a putative QTL is identified by SNP marker C2M35 on chromosome 2 and highlighted eight candidate genes in tropical *japonica* for the first time relevant to seed longevity mechanisms such as oxidative stress reduction and repair of damaged DNA consistent with previous studies suggesting their role in seed germination and longevity (Raquid et al., 2021). CPYC-type GRX encoded by *OsGrxC2.2* and a CC-type GRX encoded by *PHS9* affect germination by regulating early and late embryogenesis, respectively (Liu et al., 2019; Xu et al., 2019). Genes encoding APETALA 2 (AP2) domain containing protein (*Os02g0655200* and *Os02g0657000*) are also involved in ABA-GA antagonism during seed development and germination (Finkelstein et al., 1998; Shu et al., 2017). A gene encoding dehydrin (*Os02g0669100*) resides on the chromosome 2 reported to play an important role in seed maturation during late embryogenesis (Radwan et al., 2014). Another gene encoding a kinesin motor domain containing protein (*Os02g0645100*) regulates seed respiration was also identified that showed increased germination rate (Yang et al., 2011). Gene encoding Aldehyde Dehydrogenase7 (ALDH: *Os02g0647900*) was another interesting candidate gene which showed reduced germination rate after accelerated aging (Shin et al., 2009). Knowledge on candidate genes relevant to known seed longevity mechanism will help in improving seed longevity traits in tropical *japonica* varieties.

5. KNOWLEDGE GAP AND RESEARCH NEEDS

To understand the genetics of such complex traits, separation of QTL controlling the complex traits into individual loci is important as it help to identify approximate positions of QTL (Yamamoto et al., 2009; Miura et al., 2011). Most of the QTL for seed/seedling vigour in rice have been identified in bi-parental-derived populations and the QTL detected were located in relatively large regions and results have not been always consistent in terms of the exact locations of the QTL (Abe et al., 2012) and favourable allele/haplotype mining was not performed (Chen et al., 2019). Also, the molecular marker genotyping is time consuming and labour intensive (Chen et al., 2014). Though there is existence of large variation for seed/seedling vigour within rice gene pool, the mining of the germplasm diversity for seed vigour related QTL is still limited (Redona and Mckill, 1996; Cui et al., 2002).

Identifying QTL in segregating population exhibiting limited genetic variation and recombination with a low resolution typically in the range of 10-30 cM (Flint-Garcia et al., 2005) has made association mapping, the popularly

Table 2: Major QTL for seed dormancy and preharvest sprouting identified in rice

Sl. No.	Traits	QTL	Marker interval/ nearest marker/ position(cM)	Population	Chr. used	PV	LOD	Marker	Known QTL/ colocalizedQTL/ QTL reported in the previous studies	References
1	Seed Dormancy	*qSD-3*	RM7	F1 Population IR50 (*Indica*)/ Tachimimochi/ Miyukimochi (Japonica)NILs	3	21	9.2	SSR	Amp3, Wan et al .(1997)	Wan et al. (2005)
2	Seed Dormancy	*qSD^{s}-12*	RM270 (1 cM)	BIL (EM93-1// EM93-1/SS18-2): (11 days after ripening)	12	24.3	4.1	SSR		Gu et al.(2004)
3.	Seed dormancy	*qSD-3*	CT339-G62	DH Population (RIL)ZYQ8 (*indica*)/ JX17 (japonica)	3	14.5	5.11	SSR	C1488 Lin et al. (1998), Cai & Morishima (2000) and Wan et al. (1997)	Guo et al. (2004)
4.	Seed dormancy	*C1488*		BIL Nipponbare (japonica)/Kasalath (*indica*)//Nipponbare	3	22.5	5.147	RFLP		Lin et al. (1998)
5.	Seed Dormancy	*qDOR -11-2*	RZ141-APAGE2	RILs (Cultivated and wild rice)	11	21.8		RFLP	Pgi1, Amp3 and C, Wan et al.(1997) C1488 Lin et al. (1998)	Cai & Morishima (2000)
6		*qSD7-1*	RM21197 (-1 cM)		7	27			*qSD4*, 7-1, and 7-2 weedy rice from USA (Subudhi et al.(2012); Mispan et al. (2013)	Zhang et al. (2017)
		qSD12	RM28607 (5 cM)		12	67				

7.	Seed dormancy	*qSD5*	RM169	NILs (Cheni ahu & Kolong)	5	36.5	4.9	SSR	(*qSD5*, *qSDD5*) colocated (Lin et al., 1998; Cai and Morishima 2000; Guo et al., 2004; Wan et al., 2006).	Rathi et al. (2011)
		qSD11	RM21		11	25.4	2.9		*qSD11*, Miura et al. (2002), Guo et al. (2004) and Wan et al. (2006)	
	Duration of dormancy	*qSDD5*	RM169		5	23.4	2.9			
8.	Pre-harvest sprouting	*qPSR8*	RM447–RM3754 (1.5 cM)	(G46B/ K81) F2 population	8	43.04	26.78	SSR	*qSdn-1*, Wan et al. (2006)	Gao et al. (2008)
9.	pre-harvest sprouting		RM4108-RM5849	BILs (Koshihikari/ Nipponbare)	3	45.0	12.5	SSR		Hori et al. (2010)
	pre-harvest sprouting		RM3455-RM6905		12	10.8	3.8			
10.	pre-harvest sprouting resistance	*qSDR9.1* *qSDR9.2*	RM24039 - RM24260 Indel 2 - RM24540	CSSL Owarihatamochi/ Koshihikari (BIL s)	9 9	6.1 9.2	5.48 8.12	SSR	*qsd1* ortholog (*Os09g0433900*) (Sato et al., 2016); *OsDSG1*, Park et al. (2010)qSDR9.1(not colocalised)	Mizuno et al. (2018)
11	Pre-Harvest sprouting (Field)	*qPHS1*^{FC}	ch01-0.040 (25cM)/ ch01-0.053	Jinsang (Preharvest tolerant) and Gopum (Preharvest sensitive) RILs	1	89	35.8	SNP	*PHS9*, Xu et al. (2019)	Jang et al. (2020)

Sl. No.	Traits	QTL	Marker interval/ nearest marker/ position(cM)	Population used	Chr.	LOD	References	Marker	Known QTL/ colocalized QTL/QTL reported in the previous studies
	Pre-harvest sprouting (Chamber)	*qPHS1*^{GC}	ch01-0.040 (25cM)/ ch01-0.053		1	86.1	24.0		*Sdr6*, Marzougui et al. (2012)
	Seed dormancy (Chamber)	*qSD1*	ch01-0.040 (25cM)/ ch01-0.053		1	35.5	8.6		*qSD1.1* and *qSnd-1* are co-localized (Miura et al., 2002; Gu et al., 2006; Li et al., 2011; Lu et al., 2011; Marzougui et al., 2012; Wang et al., 2014)
12	Pre harvest sprouting resistance	*qPHS-3*^{FD} (0 cM)	KJ03_004	(Field)Odae and Unbong40 (*Japonica*) (RIL)	3	20.6	12.33	SNP	*qSdnj-3*(Wan et al., 2006) *qSD-3*(Miura et al., 2002) *qLTG3-1* (Fujino et al., 2004) *qLTG3-1* (Fujino et al., 2008) Cheon et al. (2020)
		qPHS-11^{FD}	KJ11_090 (130.91cM)		11	20.5	12.28		*qLTG-11* (Miura et al., 2001) *qSD-11* (Miura et al., 2002) *qLTG-11–1* and *-2* (Jiang et al., 2006) *qSD11BR* (Subudhi et al., 2012) *qDOR-11–4, -5,* and *-6* (Cai and Morishima, 2000) *qLTG-11* (Miura et al., 2001) *qSD-11* (Miura et al., 2002) *qLTG-11–1* and *-2* (Jiang et al., 2006) *qSD11BR* (Subudhi et al., 2012) *qDOR-11–4, -5,* and *-6* (Cai and

Sl. No.	Traits	QTL	Marker interval/ nearest marker/ position(cM)	Population used	Chr.	LOD	References	Marker	Known QTL/ colocalized QTL/QTL reported in the previous studies
							Morishima, 2000)		
		qPHS-3^{GH}*q PHS-4*^{GH}	KJ03_068 (133.21 cM) KJ04_068 (78.61 cM)	(Greenhouse)Odae	34	22.2 10.8	18.12 10.34		*qLTG-3–2* (Fujino et al., 2004) *qLTG-4–2* (Miura et al., 2001)
		qPHS-11^{GH}	OUC11_ 11310 (135.21 cM)	Unbong40 (*Japonica*) (RIL)	11	15.2	14.32		*qSDs -4* (Gu et al., 2004) qSD4 (Gu et al., 2005) *qLTG-11* (Miura et al., 2001) *qSD-11* (Miura et al., 2002) *qLTG-11–1* and *-2* (Jiang et al., 2006) *qSD11BR* (Subudhi et al., 2012) *qDOR-11–6* (Cai and Morishima, 2000)

Table 3: Major QTL for seed longevity

Sl. No.	QTL	Marker	Population interval/ nearest marker/ position (cM)	Chr.	PV	LOD	Marker used	Known QTL/ colocalized QTL/ QTL reported in the previous studies/ remarks	References
1		C2M35	RILs Azucena (*japonica*)/ Icta Motagua (*indica*)	2		2.1	SNP	New qtl	Raquid et al. (2021)
2	*qSS3.1*		Nipponbare/ 9311 BRILs			36.9	SNP	Fine mapped	Yuan et al. (2019)
3	*qSS8*	RM408-PM150	(SN265/ Lijiangxingtuanheigu) RIL	8		24.39	3.07		Dong et al. (2017)
	qSS6-1	RM439-RM494		6		53.58	2.55		
	qSS2-1	PM123-RM250		2		28.06	2.11		
	qSS3-2	RM338-RM377		3		27.71	3.94		
	qSS12-3	RM415-RM453		12		21.18	3.43		
	qSS12-2	PM155-RM511		12		44.30	10.00		
	qSS9-2	RM7424B-STS18	(SN265/Luhui 99) RIL	9		29.09	5.22		
4.	*qSS9*		NILs,Koshihikari' (*japonica*)/'Kasalath' (*indica*)//'Koshihikari	9				Fine mapped	Lin et al. (2015)
4	*qDT-SGC9.1*	S09040-S09049	Dasanbyeo/ TR22183 RILs	9	63.6	22.23	SSR		Jiang et al. (2011)
5	*qRGR-1*		Asominori / IR24 RILS	1	28.63		6.95		Xue et al. (2008)
6	*qLG-9*	R79	(Nipponbare/ Kasalath) BIL	9	59.5	13.883	RFLP	Fine mapped	Miura et al. (2002)

adapted QTL analysis approach to mine elite alleles in plants. Association mapping based on the linkage disequilibrium enables researchers to identify QTL with a higher resolution by targeting multiple alleles at individual loci, by exploiting recombination available in natural variation and by identifying association between marker and phenotype of larger number of traits (Anandan et al., 2016; Pradhan et al., 2016; Pandit et al., 2017) and has been successfully utilized dissecting several complex trait in rice (Pradhan et al., 2019, 2020; Sahoo

et al., 2020; Pawar et al., 2021). However, few studies have been carried out where QTL analysis of this complex trait have been carried out through association mapping / GWAS by using natural population (Dang et al., 2014; Chen et al., 2019; Sahoo et al., 2020).

In most of the studies related to seed vigour, RFLP/SSR markers were used (Yang et al., 2019) which are mostly distributed at low density and are not able to provide precise and complete information about the numbers and locations of QTL for the corresponding traits (Yu et al., 2011). Recently, high-density single-nucleotide polymorphisms (SNPs) are becoming more readily available owing to the development of sequencing technology (Liu et al., 2008) and mapping based on high-density SNPs show increased QTL mapping resolution, thus helping improve the efficiency of mapping (Wang et al., 2018). However, despite these obvious advantages, there were only a limited number of studies of application of SNP markers in improving seed quality traits (Yano et al., 2012; Yang et al., 2019, 2020; Chen et al., 2019).

Various QTL for seed quality related traits were reported and few already cloned (*qPHS3-2*, *qLTG-3-1*, *qSV-1*, *qSV-5c*, *qSSL1b*, *OsML1, OsML2*, *OsTPP7* and *OsIPMS1*). However, there are very few examples available for targeted improvement of seed quality traits in rice (Chen et al., 2019). Rice Genome Project (3K) with highly diverse pool carrying favourable alleles for different traits have been used for mining the various seed quality related traits/seed vigour related traits (Zhang et al., 2016, 2018). Further, association studies/GWAS in combination with functional genomics should be analysed for identification of candidate gene responsible for a trait. Sporadic report of Cui et al. (2002) and Sahoo et al. (2020) had mapped QTL for seed vigour associated with biochemical traits. Several other phytochemicals reported to be associated with seed vigour (Mahender et al., 2015; Huang et al., 2017) have not been elucidated for QTL analysis. Also reported QTL were mostly based on cultivated genotypes but only study of Jin et al., 2018 reported QTL for seed vigour from wild rice. The perennial wild species, *Oryza longistaminata* reported to be an important potential source with favourable alleles for abiotic tolerance traits along with other species of wild rice could be explored to improve seed/seedling vigour (Khush, 1997).

Numerous studies carried out for QTL mapping for seed vigour traits were not combined with expressional analysis and profiling analysis which will reduce the number of candidate gene and thus will identify the target gene (Liu et al., 2016; Wang et al., 2017).

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Speed breeding – A unique tool for higher genetic gain in rice

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SUMMARY

Plant breeders are concerned about feeding the world's expanding population. A continual stream of modern cultivars should be produced by breeding in resource efficient manner. Breeders can accelerate the crop generation or cycle in a more expeditious manner using speed breeding procedure. This chapter highlighted the current speed breeding applications in rice and exploring possibilities to integrate with modernization in breeding for faster development of lines, higher genetic gain and efficient utilization of resources compared to conventional breeding. The integration of single seed decent method with rapid generation advancement is one of the finest ways for easy adoption of this technology without any special infrastructures. The cost for breeding lines generated through rapid generation advancement is lower compared to other methods of line generation. The methods can be modified and integrated depending upon the resources, availability and investment. The rate of genetic gain can be maximized by adapting genomic predictions along with speed breeding to achieve maximum success in short time. A basic information on speed breeding and its possibilities of integration with other breeding tools must be understandable to achieve maximum success using modern breeding techniques.

Keywords: generation-advancement, speed-breeding, breeding cycle, genetic gain, genomic selection, single seed descent

1. INTRODUCTION

The most persistent task to food availability is population increment and climate change. The population growth is exerting pressure for food demand and will also be continued in future. The major challenges for high production and productivity growth are climate change, depletion of natural resources, increasing levels of CO_2, temperature, and high incidence of pests and diseases. The gradual climate change may also increase the frequency of drought and flood events in nature. Rice research has provided seventy five percent of rice varieties now grown and has increased the potential yield from 4 tonnes to more than 10 t/ha per crop. The improved rice varieties coupled with better management has been a major factor for higher production and served as foundation for providing rice supply to more than 3.5 billion people worldwide (Lenaerts et al., 2019). In general,

the yield increase per annum is reported to be approximately 1% in rice (Peng et al., 2000) and this rate may not be sufficient to meet future demands in rice. A realistic partial solution to face these challenges ahead is accelerated breeding. Therefore, it is necessary that high yielding and improved varieties can be bred faster for keeping pace with the demand of rice. Undoubtedly, the solution needs a holistic approach involving agronomic management, pest management, seed production and post-harvest technology.

A large share of yield enhancements in past can be attributed to high yielding varieties widely adopted by farmers. We require more than the average annual genetic gain of 1% in rice to meet the future requirements. Thus, development of new improved and higher yielding variety by reducing the time of the breeding cycle is one of the modest ways to increase genetic gain in crop varieties (Collard et al., 2017).

2. CONVENTIONAL BREEDING STRATEGIES

The traditional rice breeding programs around the world delivered hundreds of superior varieties to meet global food demand, however, the process and progress is slow due to longer breeding cycles. In any rice breeding program, it is required to identify suitable parents for generating crosses, selection of superior progenies for specific trait, advancement of breeding cycle, selection and evaluation of best progeny for yield and other agronomic traits and multi-site evaluation of promising and elite lines for release and notification of cultivar (Shimelis and Laing, 2012). It is interesting to note that majority of the rice breeding programmes in the world for the development of improved rice varieties have not changed in several decades (Collard et al., 2017). A large number of plants being generated from a cross in the initial generation, but these numbers are gradually reduced to a small selected number of breeding lines at the end of selection process. Nearly, 98-99% of the base population is rejected and discarded, leaving only few selected plants, on the basis of presence of desirable traits.

However, in conventional rice breeding programmes for developing new cultivars starting from generating new cross combinations and selection is followed by advancements of superior progeny until evaluation and commercial release can take more than one decade (Breseghello and Coelho, 2013). Long period is required for fixing genotypes with desired character combination. Further more complex trait is genetically, longer period it takes to get fixed. Even when complex crosses made to combine desired traits from many different parents, the period of fixation time is enhanced (Hickey et al., 2017).

3. ACCELERATED BREEDING STRATEGIES

The conventional breeders are engaged in product development take relatively long time for development of new varieties. If the desirable traits are to be in long

duration photo sensitive varieties, it would be more difficult and time consuming. Therefore, it is suggested to reduce the breeding and selection cycle in order to cope with unpredictable climate changes, resistance to new biotypes of pathogens and pests, and consumer-preferred grain quality requirements (Custodio et al., 2016). Generation time representing the bottleneck need to be resolved in the process of modern breeding approaches via fast-track research to improve productivity. The application of molecular markers in plant breeding, genetic engineering tools (Araki and Ishii, 2015), use of off-season nurseries (Shuttle breeding), and double haploid technology (Thomas and Forster., 2003) could be successfully employed in rice breeding and varietal improvement programmes to shorten the duration of breeding and selection cycles in rice.

These methodologies have got their own drawbacks; off season nurseries are expensive and there are difficulties in transportation of seed across country borders, mainly due to intellectual property related issues and biodiversity act. On the contrary, doubled haploid technology is only possible in species that are amenable to tissue culture. It is difficult to generate doubled haploid populations for *indica* subspecies as compared to *japonica* subspecies (Grewal et al., 2011). In order to overcome these difficulties, Watson et al., 2018 shorten the growth duration of a given population by short day treatment and called this approach as speed breeding. Among different methods adopted to reduce breeding cycle, speed breeding technology is proven to be realistic and effective in rapid cultivar development. Speed breeding involves growing plants under conducive growth environments to ensure early flowering and seed set as compared to normal field condition (Ghosh et al., 2018; Alahmad et al., 2018). Speed breeding is more flexible and accelerates the physiological development by growing the plants in a green house or screen house facility and several generations or cycles are completed within a shorter or quicker time (Sysoeva et al., 2010).

Rice being a short-day plant respond to light duration and light intensity during its growth and flowering stages. However, the utilization of speed breeding or forms of speed breeding in rice is very limited. The use of generation advancement strategies in rice breeding was reported in Japan and South Korea during 1960s and 1970s to overcome the duration of cold (Ikehashi and Fujimaki, 1980; Maruyama, 1989). In temperate countries rice is grown only one season per year due to severe cold. Therefore, the rapid generation advancement could be used to shorten the breeding cycle by creating artificial off-season nurseries and speed up the generation advancements for cold tolerance breeding. Generation advance technology was also popular during 1980 at IRRI for rain-fed and deep-water rice.

4. MOLECULAR METHOD INTEGRATION IN SPEED BREEDING

Integration of molecular markers for selection of traits of interest has made significant improvements in selection efficiency in varietal development programmes since last 30 years. This approach has been used as speed breeding tool for directed crop improvement strategy in rice. Molecular marker-based plant selection is often more effective than conventional methods as it increases accuracy, reduces environmental error on selection as well as saves time. Marker assisted backcross breeding (MABB) is a most common method used today to introgress target gene into a popular variety for trait improvement (Mackill et al., 2010) and this strategy has been used successfully to transfer flood, salinity and drought tolerance in rice (Gregorio et al., 2013; Ismail et al., 2013; Sandhu et al., 2017; Pradhan et al., 2019). MABB is recognized as an important tool for trait deployment and line development. The use of DNA based markers reduce the time required for varietal development and hence, increases the rates of genetic gain (Crosbie et al., 2008; Earthington et al., 2007).

Recent advances in science and technology offered cost effective genotyping with genome-wide markers which opened new avenue for selection of genotypes. Genomic selection, a new tool attracted attention of breeders for predicting genomic breeding values for a target trait (Lorenz et al., 2011). The model developed based on genome-wide markers and phenotype for a trait could be used for selecting individuals from populations based on their genomic estimates of breeding values (GEBVs) without phenotyping. Thus, such efficient genomic selection approach can support the speed breeding for rapid generation advancement and it would be an efficient strategy to optimize cost effective breeding approach (Hickey et al., 2019). Ohnishi et al. (2011) combined the genomic selection and speed breeding to enhance the rate of genetic gain in rice breeding and accelerate the generation cycle. Both genomic selection and speed breeding could reduce the generation time and enabling indirect selection for target key traits. Genomic selection allows for accurate selection of complex traits like yield and to shorten the breeding cycle which ultimately increase the rate of genetic gain (Heffner et al., 2010). Watson et al. (2019) developed multivariate genomic selection model and correlated speed breeding traits with field evaluated traits, which indicated higher selection intensity under speed breeding and increased genetic gain than direct field phenotyping. Application of advanced breeding tools like genomic selection along with speed breeding approach enhanced the genetic gain per generation and per unit cost in rice improvement programs. Thus, the concepts of speed breeding, its applications, role in genomic selection for improving the genetic gain in rice breeding programs encouraged breeders to integrate this tool as a new breeding strategy.

5. OPPORTUNITIES OF SPEED BREEDING IN RICE IMPROVEMENT

Rice being annual crop more sensitive to photo-period and varied temperature levels during its growth and reproductive stage. An average time period in rice from seed to seed is around 120-140 days and at a maximum of two generations can be completed in a year. If the desirable traits are to be in long duration photo sensitive varieties it would be more difficult and time consuming. Under such circumstances nearly ten years are required to develop a new variety. Speed breeding offers the opportunity to develop fixed homozygous lines and could accelerate the procedures in the development of a new variety. It is now possible to increase the number of generations per year by manipulating photo-period, temperature, soil moisture, hormone application and plant density (Wanga et al., 2021). Combining the speed breeding protocols with modern genomic tools like marker assisted selection, genomic selection and next generation sequencing technologies results in fast-track development of new superior varieties in rice. Rana et al. (2019) integrated speed breeding techniques with marker aided backcrossing program resulted in faster development of new salt-tolerant variety. Jamali et al. (2020) proposed "speed DUS testing" which is a speed breeding model for DUS testing, and extended the utility of speed breeding from varietal development to varietal protection.

6.CROP ENVIRONMENT MANAGEMENT FOR REDUCING BREEDING CYCLE IN RICE

Rice is a short-day plant and sensitive to duration and quality of light. The light duration protocols developed for speed breeding benefits sustained photosynthesis and flowering regulation all the year (Bhatta et al., 2021). The lighting systems with far-red rich wave lengths regulate early flowering whereas, the low levels of red-blue wave lengths known to cause suppressed stem elongation thereby reduce the plant height (Jähne et al., 2020). The longer day length of 14 hours light and 10 hours dark in early stage of crop growth accelerates vegetative development followed by short day length of 10 hours light and 14 hours dark promotes flowering in rice. These modifications in lighting system have been reported to achieve four breeding generations in the span of 11 months (Rana et al., 2019). Jähne et al. (2020) reported effective use of LED lights coupled with solar power systems for regulating light duration and quality for producing five generations a year in rice which is also a cost-effective approach. Apart from the quality of light, the temperature also plays a major role in flowering and seed set in rice. Temperature maintained at 25 ± 1 °C can facilitate flowering and seed set; however, temperature of 33 °C and above causes reduced pollen sterility in rice (Singh et al., 2015). Therefore, well maintained temperature within the critical limits can advance flowering and seed set in rice under speed breeding.

High density planting results in long and lanky plant growth due to competition among plants for light source, leading to advancement in transition from vegetative to reproductive stage (Warnasooriya and Brutnell, 2014). This helps in increasing the number of generations per year by inducing early flowering. Planting 400 plants per m^2 (5x5 cm) reduced crop cycle up to 40 days in rice (Rahman et al., 2019). High density planting is one of the low-cost speed breeding strategies suitable for maintaining large populations. Besides high density planting increased CO_2 level also enhance the speed of transition from vegetative to reproductive stage and induce early flowering (Jagadish et al., 2016). Increased CO_2 level of 350/700 reduced the days to flowering by 7 days (Springer & Ward, 2007). Tanaka et al. (2016) documented that the rapid generation advancement and high level of CO_2 reduce root growth and days to flowering, thereby, help in reducing the generation time in rice.

7. SPEED BREEDING USING RAPID GENERATION ADVANCEMENT (RGA)

Popularization of speed breeding for generation advancement is associated with phenotypic methods. Cutting-edge inventions in molecular biology, marker technology and phenotyping strategies can be successfully integrated with speed breeding. The perfect combination of speed breeding with effective selection strategy for a target trait will maximize the genetic diversity and efficiency of breeding program (Johnston et al., 2019). After generation of crosses from selected parents, it is required to fix the progenies to a homozygous state before selection of superior progenies. Speed breeding have been a better option for reducing generation time and advancing the generations before actual selection of progenies. The approach depends on manipulation of planting density, photoperiod, soil nutrition, light intensity etc. which induces the plant to produce early flowering and complete its life cycle by setting seeds. Following this approach, it is possible to complete 3 to 7 generations per year in rice breeding. Different crops have different photoperiod requirement for flower production and seed setting (Saito et al., 2009), thus explaining the importance of light duration and quality to promote flowering in any crop.

7.1 Compatibility of single seed decent (SSD) method with RGA and speed breeding

Traditional selection methods require genetically stable population for selection and need long inbreeding cycles; hence these methods are not ideal for speed breeding approach. Most appropriate method of selection, compatible with speed breeding in rice is single seed descent method (SSD). This method involves continuous selfing of segregating populations and harvesting of single seed from every F_2 plant and advanced to next generation. Each line developed by SSD can

be traced back to its corresponding F_2 plant (Fehr, 1991). SSD method requires less growing area and generation can be advanced under high density planting conditions which is helpful to adopt speed breeding (Arbelaez et al., 2019). Tanaka et al. (2016) used SSD method for rapid generation advancement method in growth chambers and developed popular cultivar, Nipponbare. Among the selection procedures SSD method was found the best selection method for speed breeding to carry out indoor (control environment) and in field conditions followed in rice breeding programs. The field-based method in rice involves growing of plants under both direct seeded and transplanted conditions. The method has to be optimised depending upon the resource availability. At least, 2000 F_2 progenies to be advanced in SSD which is an ideal population size for higher genetic recombination and genomic selection. A landmark study Fahim et al. (1998), who compared F_6 breeding lines developed by pedigree, bulk, modified pedigree and SSD using two breeding populations and made a conclusion that none of the best lines derived from pedigree, bulk or modified pedigree were significantly better for any trait than the lines produced using SSD. In fact, some of the best lines for grain yield were derived from the SSD method. Although, 'poor' lines are carried forward during the line fixation process in RGA but, the cost of line fixation associated with RGA is much cheaper that it will adjust the budgetary burden of bringing poor performing lines forward. Finally, a larger number of good lines with low cost per line would be achieved. The cost for breeding lines generated through RGA is estimated to be significantly lower compared to double haploids (Lenaerts et al., 2019).

7.2 Resource optimization for RGA

The RGA can be implemented depending upon the resource availability and facilities such as screen houses or greenhouses to manipulate growing conditions under controlled environment. Under less resources, RGA can be performed directly in the field (Field RGA) if the annual temperatures permit for continuous sowing and planting of rice. Compared to DH lines, the RGA generated breeding lines are estimated to be significantly of lower cost compared to doubled haploid (Lenaerts et al., 2019). The field based RGA can be manipulated by direct seeded single seed, direct panicle sowing and high-density transplanting (5x5 cm) to make it reliable and effective.

8. GENETIC GAIN UNDER SPEED BREEDING

Genetic gain is referred to as an amount of increased performance achieved annually under artificial selection. It is measured by a difference in performance between selected population and its progenies in the succeeding generation. Estimation of genetic gain is important to analyze strength and weakness of breeding plans and plan for future (Fufa et al., 2005). A method to maximize the

genetic gain under inbreeding was developed by Woolliams et al. (2015). Expected genetic gain generally defined as "$G = i\, r\, \sigma_A / t$, where, "G represents the response to selection with intensity i, accuracy r, standard deviation of breeding values σ_A and breeding cycle time t. Advances in rice breeding in the genomic era accompanied by advanced management strategies contributed to achieve 0.8-1.2% genetic gain in rice production (Li et al., 2018). However, this rate of success is not enough to meet the targets of rice production by 2050 and in order to meet the targeted rice production it is required to achieve 2% genetic gain by adopting innovative and modern breeding tools in a strategic way. Several breeding approaches have been employed to achieve balanced genetic gain in short-term and long-term breeding plans. Genomic selection provides appropriate way to maximize the genetic gain with estimate of GEBVs of genotypes in the population (Akdemir and Isidro-Sánchez, 2016). The selection of parents for higher GEBVs and crossing of the high GEBVs parents supposed to generate an ideal population.A population consisting of at least 2000 progenies could be advanced from F_2 to F_6 in a quicker time through RGA using SSD method would provide an ideal recombination and increase the rate of genetic gain. The yield advantage of genetic gain over years is very important for assessing the progress of breeding programs in rice. Application of genomic selection for indirect selection of trait performance purely based on genomic values increases the accuracy of selection and adds to increased genetic gain among the progenies which intern increases the yield advantage in quantifiable units. The rate of genetic gain can be maximized by adapting genomic predictions along with speed breeding to achieve maximum success in short time. The concepts of speed breeding and genomic selection are complementary for selection of appropriate genotype and advancing it further thereby achieving accelerated genetic gain. Voss-Fels et al. (2019) standardized a genomic selection model and named it as "SpeedGS" for combining genomic selection with speed breeding. The activities under this system works as fast-track breeding programs to reach the goal of achieving higher yield targets in rice breeding. The success of higher genetic gain relies on heritability of trait and length of breeding cycle of crop, for which speed breeding and genomic selections got potential deal and enhance the genetic gain per unit time. Thus, speed breeding combined with advanced breeding tools like genomic selection offered new avenue to achieve higher genetic gain in rice breeding programs.

9. CONCLUSION

The overall rate of genetic gain is a time constraint approach. The speed breeding strategy is successfully employed for advancing the generation of a cross rapidly within a short period of time. It helps in producing a large number of fixed progenies per unit land and time. As selection is not practiced during advancing of generations which permits the accumulation of major genes and thousands of

minor genes in the fixed progenies. Therefore, it is concluded that the speed breeding approach is not only cost effective, but also increases the rate of genetic gain for yield by improving the effectiveness and efficiency of breeding operations in rice.

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Doubled Haploid Technologies for Rice Improvement: Technical Progress and Prospects

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SUMMARY

Rice yield needs to be improved in terms of quantity to feed the increasing population. The limitations in rice production and productivity necessitate the integration of biotechnological tools with traditional breeding strategies. The efficiency of anther culture has been demonstrated by the instantaneous fixation of homozygosity by diploidization of regenerated haploid plants and offers an effective pathway for improvement of rapidly evolving inbred lines. Despite the fact that anther culture has been shown to be effective in speeding up breeding in a variety of crop species, including rice, associated limitations still prevent it from reaching its full potential. While anther culture is widely used in the breeding of *japonica* rice, its use in *indica* rice is limited due to recalcitrant genetic backgrounds. Several factors influence the performance of anther culture and restrict the efficiency of androgenesis. Early anther necrosis, slow callus response and proliferation, and low green plant regeneration have all been identified as restrictions, as has the albinism associated with *indica* rice, which has been thoroughly resolved by ICAR-NRRI, Cuttack. This chapter describes different aspects of doubled haploid production based on different techniques such as gynogenesis, androgenesis, and the most novel Haploid induction line and the applicability of DHs as a complementary tool for rice improvement.

Keyword: diploidization, homozygosity, japonica rice, biotechnological, doubled haploid

1. IMPORTANCE OF DOUBLED HAPLOID IN RICE BREEDING

Doubled-haploid breeding has outmanoeuvred the traditional breeding methods and emerged as an exciting and powerful tool for crop improvement (Purwoko et al., 2010). Shortening breeding period due to immediate homozygozity fixation, high selection performance, increased genetic diversity, and expression of desirable recessive genes are few of the several benefits established through the doubled haploid breeding technique (Devaux and Pickering, 2005). Considering the benefits of DH technology in many crops, it has not been used to its full potential in rice, despite the fact that rice is a staple food for more than half of the world's

population. Furthermore, the production of high-yielding rice cultivars is critical to meet the demands of an ever-increasing population as well as the challenges that come with it and that to within limited time frame.

In vitro doubled haploids are attained by, gynogenesis, a method of producing haploid embryos from unpollinated flower parts including ovules, placenta-attached ovules, and whole flower buds (Murovec and Bohanec, 2012) or androgeneisis, governed by the *in vitro* culture of anther or derived microspores on specific media. Chang (1984) used unhusked young flowers from Zao Geng No.19 (*japonica* rice) in liquid medium to obtain gynogenic haploid (77.5%) and diploid (22.5%). In anther study, Dong-ling et al. (1988) used unfertilized ovaries of Hubei Photosensitive Genic Male-sterile Rice (HPGMR) cv. Nongken 58 and recorded the highest callus induction frequency to be 3.33%. Although, gynogenesis provides a source of haploid development in plants with a high male sterility rate (Bhat and Murthy, 2007) in many crop species but the most critical constraints have been poor regeneration and low haploid production along with the high frequency of heterozygote contaminants developed from the gynoecium wall.

Anther culture, on the other hand, has been used to grow a number of varieties and improved breeding lines, mainly in *japonica* cultivars (Grewal et al., 2011). *Indica* rice cultivars, on the other hand, have weak androgenic response, making this technique ineffective. Furthermore, early anther necrosis, low callus proliferation, and albino-plant regeneration are some of the major issues that have been experienced and require immediate attention for its widespread usability in *indica* rice. As a result, efforts are being made to address low anther culturability by analysing several main factors that influence anther culture performance (Trejo-Tapia et al., 2002; Jacquard et al., 2006). Rice breeding operations could be speed up and cost-effective if DH technology were combined with phenomics and genomics. Niizeki and Oono were the first to report on developing haploids through anther culture in rice (1968). Following that, in many countries, the doubled haploidy method, combined with traditional breeding, resulted in the production of many rice varieties with pest and disease tolerance, high yield, and good grain quality. In addition, anther culture can aid other biotechnological approaches such as gene transformation technology and QTL discovery.

Amidst all of the benefits of DH technology, it has not been widely applied in *indica* rice to fully exploit its potential. This is due to a lack of experience as well as the variable responses of various genotypes in *in vitro* culture. Given the release of a salt-tolerant *indica* variety through anther culture (Senadhira et al., 2002), the capacity of anther culture for *indica* rice breeding is not completely exploited. Some of the issues encountered include early anther necrosis, low callus

proliferation, and albino-plant regeneration are in need of immediate attention. To achieve success in rice anther culture, several influencing factors (Fig. 1) associated with poor androgenic response was discussed in this chapter. While one-step androgenesis (somatic embryogenesis) is less expensive than two-step organogenesis, the latter has become more common in rice due to its responsiveness.

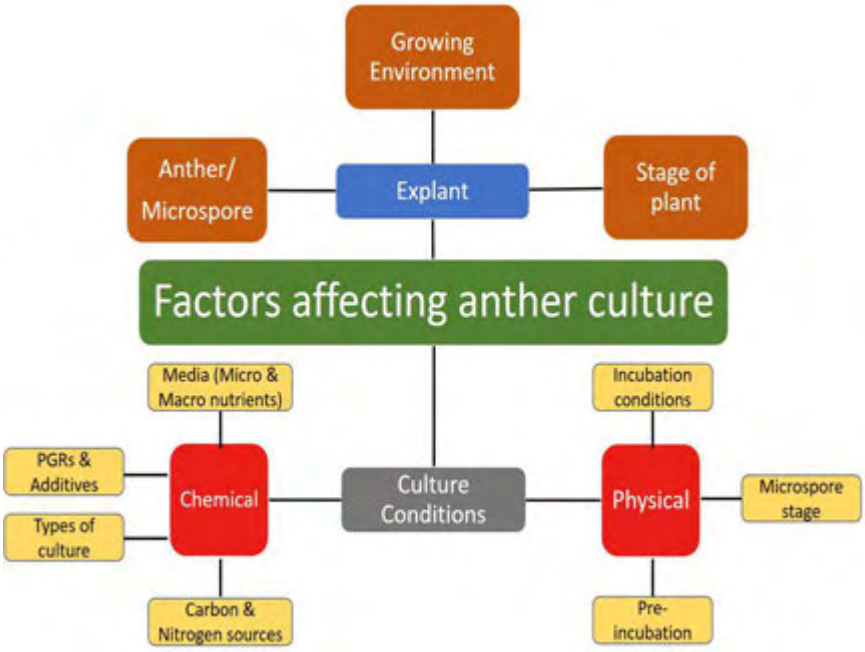

Fig. 1. Factors affecting anther culture in *indica* rice

2. RICE ANTHER CULTURE: AN OVERVIEW

The physiology of the donor plant is a significant factor in rice anther culture's success. Anthers collected from panicles grown in the field performed significantly better in anther culture than anthers collected from pot plants in the greenhouse (Veeraraghavan, 2007). Next, the microspore stage is thought to play a role in androgenic reaction. The required stage of the microspore is identified using an easily detectable morphological feature of the plant that has a strong connection with the pollen production stage (Nurhasanah et al., 2015). The gap between the flag leaf's collar and the ligule of the tiller's penultimate leaf is usually a good indicator of anther maturity. The late uni-nucleate to early bi-nucleate stage of microspore production has been identified as the most suitable stage. However, stage as early as mid-uni-nucleate, was identified as required microspore stage for effective androgenic response (Fig. 2).

Androgenesis *in vitro* is influenced by a variety of chemical and physical influences. Low-temperature shock with sufficient length is the most commonly

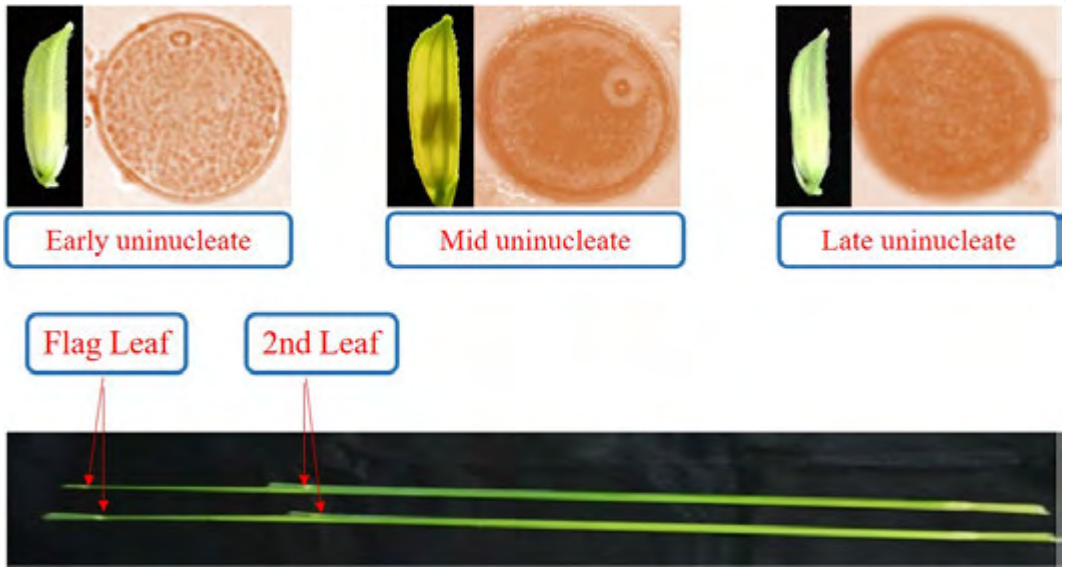

Fig. 2. Morphological indicator corelating to microspore stages used in anther culture

used pre-treatment for androgenesis. Mishra et al. (2013) investigated the effect of cold pre-treatment at 10 °C for 7–9 days on Rajalaxmi (CRHR 5) and Ajay (CRHR 7) anther culture responses, finding that cold pre-treatment had a positive effect on callus induction frequency regardless of the media or plant growth regulators (PGRs) used; however, prolonged treatment beyond the optimal duration inhibited androgenesis. However, Naik et al. (2017) reported, the cold treatment (10°C) for 8 days was found to be successful for callus induction and green plant regeneration in *indica* rice hybrid, BS6444G. Furthermore, the efficacy of androgenesis in a long-duration indica rice hybrid was influenced by a two-day pre incubation cycle at 10°C (Rout et al., 2016).

N6, MS, B5, and Potato-2 medium are the most widely used basal media for anther culture. Following that, by changing nitrogen concentrations and sources, carbon content and sources, and changes in vitamins and their concentrations, several media (MSN, SK1, He2, and RZ) were formed based on N6 media, which were found to be promising for anther response in rice. After a trial with 16 different media, Min et al. (2016) discovered the best media for androgenesis in the generation of DHs from F_1s of two inter-varietal crosses in terms of callusing (N6) and shoot regeneration (½MS). However, in *indica* rice hybrids, two basal media, N6 and MS, were found to be successful for callusing and green shoot regeneration, respectively (Rout et al., 2016; Naik et al., 2017).

Given the importance of plant growth regulators in tissue culture, the effects of various PGRs on androgenesis were investigated. In spite of the fact

that 2,4-D has been shown to be a potent source of auxin for callus induction from cultured anthers, lower 2,4-D rates were found to be more efficient for calli regeneration in *indica* rice than higher 2,4-D rates (Fig. 3). In 7–10 days after being transferred to media supplemented with NAA (0.5 mg/L), BAP (1.0 mg/L), and Kn (1.0 mg/L), the calli formed green spots, and adequately supported the green shoot regeneration from subcultured calli. Auxin type and concentration appear to influence microspore growth, with 2,4-D promoting callus formation and IAA and NAA promoting direct embryogenesis (Ball et al., 1993). In the generation of DHs from F_1s of two inter-varietal crosses and hybrid rice, 2,4-D was found to be effective for callus response, while NAA, Kn, and BAP combinations showed shoot regeneration (Min et al., 2016; Rout et al., 2016; Naik et al., 2017).

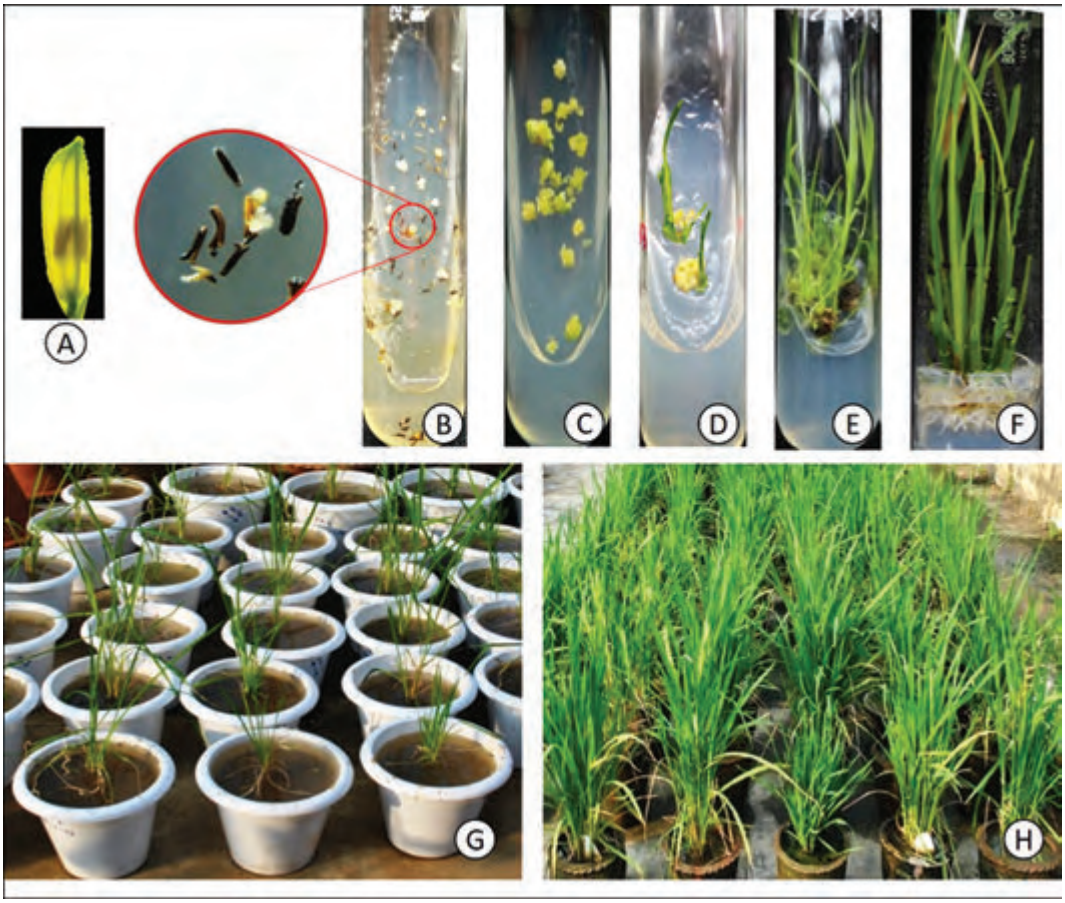

Fig. 3. Schematic representation of *indica* rice anther culture

In androgenesis, the nitrogen content of the culture media (in the form of nitrate and/or ammonium ions) is essential. Nitrate (NO_3) to ammonium (NH_4^+) ratio has been found to be a key factor in the success of anther culture and the *in vitro* induction of embryogenic calli in *indica* rice (Grimes and Hodges, 1990). The removal of total nitrogen from media, resulted in limited proliferation and shoot development, but higher regeneration was observed in media containing NO_3 as the sole nitrogen source (Ivanova and Van Staden 2009), while replacing NH_4^+ with NO_3 reduced the rate of hyper-hydricity. Herath et al. (2007) demonstrated that altering three different media (N6, B5, and Miller) with one-half the rate of NH_4^+ and double the rate of KNO_3 as nitrogen source increased the frequency of callus induction.

Because of osmotic and nutritional effects, a carbohydrate source is needed for androgenesis. In BS6444G anther culture, a popular *indica* rice hybrid, Naik et al. (2017) demonstrated the superiority of maltose over sucrose as a carbon source for callusing. High-intensity white light inhibits embryogenic induction of microspores, while darkness or low-intensity white light is found to be encouraging in anther culture (Bjornstad et al., 1989). On occasions, it has been discovered that continuous incubation of anthers in the dark is needed for *indica* rice anther culture.

The majority of *in vitro* androgenic responses are genotype dependent. In general, *indica* rice cultivars have a lower androgenic response than *japonica* rice cultivars. Even among *indica* cultivars, there is significant variation in microspore callus and green shoot regeneration (Rout et al., 2016; Naik et al., 2017). The most non callus-responsive cultivars have the highest regeneration frequency, while the most callus-responsive genotypes have the lowest regeneration potential. As a result, picking just one move, such as callus improvement or shoot regeneration, may not be enough to create an effective androgenic process. Therefore, identification of genotypes bearing both the traits is critical for overall anther culture process improvement.

The cost-effectiveness of spontaneous genome doubling has been demonstrated in a variety of cereals, including rice. According to Segu-Simarro and Nuez (2008), rice anther culture has 50–60% of autoduplication. Surprisingly, a recent study on the rice hybrid BS6444G (Naik et al., 2017) and 27P63 (Pattnaik et al., 2020) revealed 90-99 % spontaneous doubling. The rate of doubling varies greatly between genotypes (Chalyk, 1994; Kleiber et al., 2012). Endomitosis is a form of chromosome doubling that occurs without the nucleus being divided. In general, haploid cells are unstable in culture, with a proclivity for endomitosis. This property of haploid cells is used to create homozygous plants by diploidization. A small portion of haploid plant (explant) is grown in a suitable medium with

growth regulators as part of the procedure (auxin and cytokinin). This results in the development of calluses, which are then differentiated and endomitosis causes chromosomal doubling during callus formation. As a result, diploid homozygous cells and, eventually, true homozygous plants are formed.

The presence of a large proportion of albinos among regenerated plants after anther culture is the most frustrating aspect of *indica* rice androgenesis, and it remains a significant barrier to its application in rice breeding. Albinos can be found anywhere from 5% to 100% of the population (Talebi et al., 2007). The prevalence of albinos is affected by a number of factors, including pre-treatment conditions of culture medium, and could be significantly reduced by shortening the culture time (Asaduzzaman et al., 2003). In regeneration, albinos (60–100%) were found to be detrimental for maximising an androgenic response. The existence of large-scale deletions in the plastid genome derived from the albino haploid plants of *japonica* x *indica* hybrids anther culture (Yamagashi, 2002) and the absence of such deletions in green regenerants seemed to indicate that plastid genome played a role in deciding the albino phenotype. As a result, India's ICAR-NRRI attempted to establish a protocol for albinism suppression, which resulted in the standardisation of a 100% albino-free shoot regeneration system in *indica* rice (patent filed 1355/KOL/2015 titled "Method for albino-free shoot regeneration in rice via anther culture"). For rooting, the well-developed shoots were transferred to MS media containing 2.0 mg/L NAA, 0.5 mg/L Kn, and 5% sucrose. After 7 days of culture, root induction begins, followed by profuse rooting after 4 weeks.

3. CHARACTERIZATION OF REGENERANTS AND PLOIDY ANALYSIS

To differentiate doubled haploids from redundant heterozygous diploids grown in the net house, evaluation of regenerants is needed after successful callus induction and regeneration. Various undesired heterozygous plantlets can be obtained during the development of homozygous lines. These contaminants can be regenerated from the somatic tissue of inoculated plant organs including anther wall cells, tapetum, and filaments in anther culture. Therefore, before using putative haploids and doubled haploids, a reliable and fast selection of true regenerants is needed.

Variations in nuclear DNA content of cultured plant tissues resulted in ploidy modifications, which changed the phenotypic characters of the regenerants, which were classified as triploid, tetraploid, polyploid, or aneuploid. Agronomic traits and ploidy levels of rice plants were affected by chromosome aberrations (Zhang and Chu, 1984). Ploidy shifts, such as deletion, duplication, and rearrangements, were particularly common in chromosome number variations

in regenerated plants (d'Amato, 1989). The established calli may be of mixed form, such as haploid, diploid, triploid, and tetraploid, because the isolated anthers are often contaminated by the anther wall tissues (Dunwell, 2010). Several methods can be used to assess different ploidy statuses.

3.1 Morphological

By morphological examination, fertile diploids can be differentiated from other ploids such as haploids, triploids, and polyploids (Fig. 4). Putative DHs and diploids have a normal morphological appearance and 70–80% grain fertility, while the polyploids are tall, wide, and have long, thick leaves, with less than 1% spikelet fertility. Haploids are confirmed by their short size and lack of spikelet fertility. Mixploids are sometimes found in regenerants, which can be distinguished after grain formation and maturity.

Fig. 4. Ploidy status in anther derived regenerants

3.2 Cytological

The classical cytological approach was thought to be the best tool for determining ploidy status out of the many methods evaluated previously. The chromosomes of root tip cells arrested in metaphase stage can be counted to determine ploidy status in plants (Mishra et al., 2015). This method, however, is repetitive and time-consuming, and also necessitates expertise. However, tissues containing dividing cells may not always be available for study.

3.3 Flow cytometry

Flow cytometry is the most accurate tool for determining ploidy level (Cousin et al., 2009; Wang et al., 2016) since nuclear DNA content and ploidy level are

closely related. It is widely used in plant ploidy analysis. It's commonly used to determine the ploidy degree of individuals collected from haplo-diploidization or chromosome doubling experiments (Grewal et al., 2009; Ochatt et al., 2009). This approach was used to determine the ploidy status of the regenerants derived from the original.

3.4 Molecular markers

Neither of these methods were able to distinguish true DHs from heterozygotes (somatic tissue-derived diploids), suggesting that molecular markers could be the best choice for identifying true DHs (Rout et al., 2016; Naik et al., 2017; Pattnaik et al., 2020). Genomic molecular markers are extensively heritable, and also have large number of variants, which can often distinguish closely related genotypes. Simple sequence repeats (SSRs) are abundant and widely distributed throughout the rice genome. Hence, SSRs are the most widely used markers in plant derivatives for determining the source of origin (microspore mother cells or diploid somatic tissue embryogenesis), parental contribution (allelic frequencies), and homozygosity. In rice hybrids, SSRs have been used to successfully distinguish homozygous spontaneous doubled haploids. (Fig. 5)

4. APPLICATION OF DHS IN RICE IMPROVEMENT

The DH technique was used to solve *indica* rice hybrid constraints, such as: (1) costly seed, which prevented Indian marginalized farmers from using the seed every season; and (2) erratic environmental conditions and non-synchronized flowering. Implementation of DH technology in rice hybrids (PHB71 and KRH2) resulted in development of Satyakrishna (2008) and Phalguni (2010), which were released as varieties. Application of androgenic protocols in two more *indica* rice hybrids, CRHR32 (an elite long-duration *indica* rice hybrid developed at NRRI,

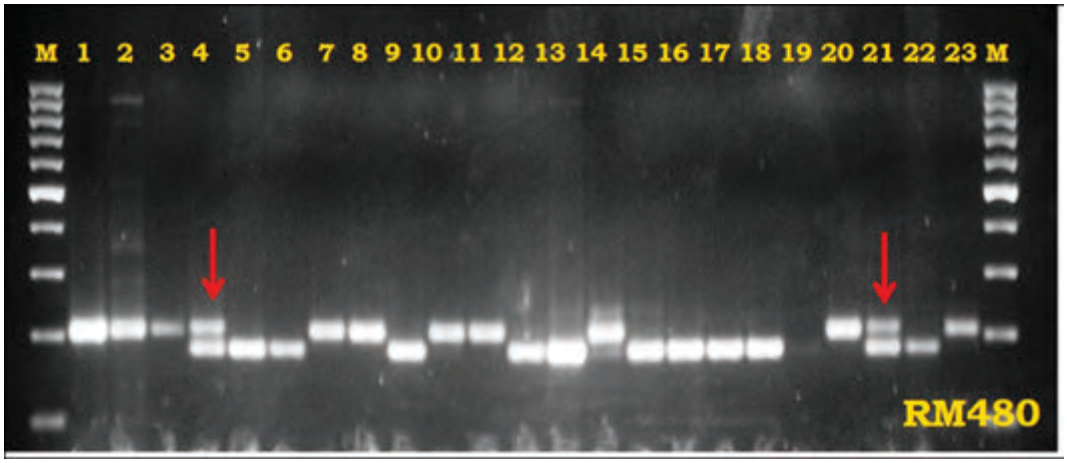

Fig. 5. Discrimination of heterozygous diploids from DHs using SSR marker

Cuttack) and BS6444G (a popular rice hybrid, Bayer Seed Pvt. Ltd.), produced a large number of DHs (Rout et al., 2016; Naik et al., 2017). The grain yield of some of the DHs was comparable to that of the parent rice hybrids, and the grain quality of the DHs was also superior to that of the hybrids.

4.1 Mapping of genes and QTL for biotic and abiotic stress

A quantitative trait locus (QTL) is a genomic region influenced by multiple genes that contributes to distinct traits. QTL are the primary determinants of essential agronomic traits in plants, and mapping these genomic regions to particular chromosomal locations is critical for crop improvement. Doubled-haploid populations have their own importance among the various mapping populations used to map QTL in plants. Since QTL effects are small and largely affected by environmental factors, precise phenotyping with repeated trials under various environmental conditions is needed. Consequently, DH populations are one of the few mapping populations of true breeding nature, allowing for successful QTL mapping in plants. In comparison to other non-segregating, true-breeding populations including near-isogenic lines (NILs) and recombinant inbred lines (RILs), DH mapping populations are formed in a short period *i.e.*, in one generation. As a result, DH populations are an accurate and efficient source for gene and QTL mapping (Forster et al., 2007). Therefore, DH breeding by androgenesis has emerged as a powerful method and a viable alternative to other crop improvement techniques (Germana, 2011).

For the identification of salt tolerance QTL/genes, anther culture was used to establish a mapping population from Savitri (a high-yielding *indica* rice variety) x Pokkali (a salt-tolerant *indica* rice genotype). A systematic analysis of 117 DHs derived from Savitri x Pokkali F_1s identified four candidate genes for salinity tolerance at the germination stage: LOC_Os01g09550 (apical meristem protein), LOC_Os01g09560 (mitochondrial processing peptidase subunit alpha), LOC_Os12g06560 (putative protein), and LOC_Os12g06570 (cyclic nucleotide-gated ion channel) (Fig. 6).

In rice, efforts are also being made to map QTL that contribute to viral and bacterial disease resistance. The DH population derived from the IR64 x Azucena and IRAT177 x Apura, crosses mapped a QTL responsible for rice yellow mottle virus resistance on chromosome 12 of the rice genome (Ghesquière et al., 1997). To recognise rice blast resistance genes, researchers used a DH population derived from a cross between the blast-resistant variety Zhai Ye Qing8 and the susceptible variety Jin Xi17, coupled with cDNA amplified fragment length polymorphism and bulked segregant analysis (Zheng et al., 2004).

DH lines are also used to map QTL for insect resistance, in addition to rice diseases. Six QTL linked to brown planthopper (BPH) resistance in rice were

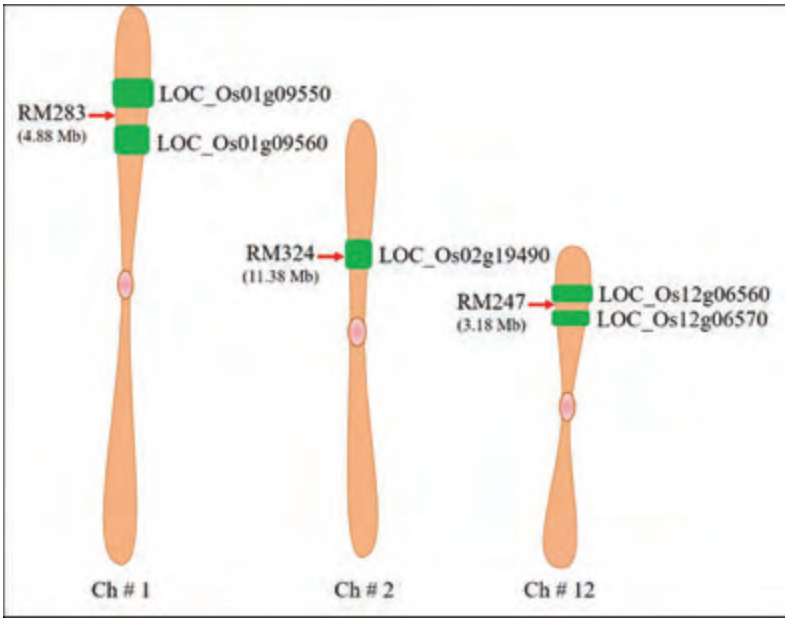

Fig. 6. Map of salt tolerance QTL/genes in indica rice using DH derived population

mapped using DH lines formed by crossing IR64 and Azucena (Soundararajan et al., 2004). Geethanjali et al. (2009) identified QTL responsible for white backed planthopper (WBPH) resistance using the same DH population. Eventually, QTL for WBPH resistance were identified and mapped using a DH population derived from a cross between Cheonhcheong and Nagdong. A DH line showing resistance to both insect pests was developed using an F_1 rice line derived from a cross between JSNDH13 (BPH resistant) and CNDH32 (WBPH resistant) (Yi et al., 2015).

4.2 Development of improved lines and rice cultivars

Anther culture technology can fix all the segregating loci (100%) in one generation, compared to eight to ten inbreeding generations with the traditional approach (Purwoko et al., 2010). Moreover, it is capable of preserving maximal genetic variation and heritability (Prigge et al., 2012), allowing for the early selection of transgressive segregants/superior genotypes. Anther culture provides robustness for early-selection outcomes, making trait improvement strategies more effective with lesser time (Dicu and Cristea, 2016). Therefore, it is a financially viable choice for accelerating breeding with increased selection accuracy and breeding value (Rober et al., 2005). In a variety of crops, including rice, doubled haploids have been approved for commercial use. DHs are also used as parents in the production of rice hybrids (Mishra and Rao, 2016). In rice, China, India, Japan, South Korea, Hungary, and the United States have produced and published over 100 rice breeding lines or varieties (Siddique, 2015) (Table 1).

Table 1 Commercialized rice doubled haploids and country of origin.

Country	Name of DH	Important features	Reference
India	Satyakrishna (CR Dhan 10) Phalguni (CR Dhan 801)	High yield, higher quality	NRRI (2008, 2010)
China	Huayu I,Huayu II,Xin Xiu, Late Keng 959, Tunghua1, Tunghua 2, Tunghua 3, Zhonghua 8, Zhonghua9, Huahanzao, Huajian 7902, Tanghuo 2, Shanhua 7706, Huahanzao77001, Tanfeng 1,Huayu III, Ta Be 78, Guan18. Huayu 15, Milyang 90, Hwacheongbyeo, Joryeongbyeo, Hwajinbyeo	High yield, superior grain quality, resistance to blast and bacterial blight diseases, resistant to lodging and diseases, Resistantto BPH, stripe virus disease, resistant to tenui-virus	Zhang (1989); Hu and Zeng (1984); Chen et al. (1986) Shouyi & Shouyin (1991); Chung (1987); Lee et al. (1988)
South Korea	Nanhua 5, Noll, Hua 03, Guan 18	Early maturity, good quality, and disease resistance	Zhu and Pan (1990)
Philippines	AC-1	Salinity tolerance	Thomson et al. (2010)
Japan	Joiku No. 394, Hirohikari, Hirohonami AC No. 1, Kibinohana	High yield, quality type, cold resistance	Sasaki et al. (1988); Maeda et al. (1990)

4.3 Mapping populations for essential traits

Rice is a major source of energy while a low supplier of protein, thiamine, riboflavin, niacin, and micronutrients (Fe, Zn, Ca) in the diet (Juliano, 1997). However, it is unable to provide the country's nutritious food protection due to a lack of essential micronutrients, which could be termed as "hidden hunger". Fe and Zn shortages are among the most severe micronutrient deficiency conditions, not just because of their serious health effects, but also because of the large number of people affected worldwide. As a result, enriching rice grain with iron and zinc is expected to have significant health benefits for rice dependent population. Furthermore, the presence of a significant amount of phytate (0.06-2.22%) in rice, which prevents the absorption of Fe and Zn (Liang et al., 2007), must be reduced. As a result, biofortification of rice varieties with higher protein, iron, and zinc content and lower phytate content could be targeted for rice improvement. Rice nutritional value can be improved in two different ways. The first method is to take advantage of genetic variation that can be obtained by breeding or genetic modification. The doubled-haploid method is another promising technique, in which fixed genetic materials can be produced faster

than other mapping populations and easily analysed through years and locations. This approach produces less hereditary abberations than conventional breeding methods, making it a valuable genetic resource for mapping QTL/genes for biofortification traits. DH populations are useful in detecting QTL for micronutrient concentrations in rice grain in several studies. QTL for Agronomic traits and grain micronutrients were recently mapped using two DH populations developed by IRRI (Swamy et al., 2018). In these DH populations, significant genetic variation has been found for all traits, which can be used as donors in breeding programmes or directly evaluated in multi-location trials to assess their performance further.

4.4 Development of CMS and Haploid Inducers

DH aids in the creation of new cytoplasmic male sterile (CMS) lines for the production of F_1 hybrid seed. Pollinating this Haploid Inducer (HI) line with different inbred lines produces paternal haploids that bear CMS if the HI line has a CMS history. To create a new diploid CMS line, one or a few paternal haploids must be pollinated with pollen from the maternal inbred. For cytoplasmic conversions, using paternal haploids has three distinct advantages: (1) reduction in development cycle (2 generations), (2) the new CMS line has 100% of the genomes of either of the inbred lines, and (3) chromosome doubling is not required (Weber, 2014). For a long time, this approach has been used in maize using the *ig1* system (Evans, 2007).

4.5 Reverse breeding

Hybrid seeds are created by crossing two inbred lines. Reverse breeding, as suggested by Dirks et al. (2009), is a novel plant breeding technology that can directly produce parental inbred lines from any hybrid. A three-step pathway was proposed for reverse breeding: (1) inhibiting meiotic crossover in F_1 plants to produce gametes with non-recombinant parental chromosome combinations, (2) DH line generation through in vitro unfertilized ovule or anther culture, and (3) regeneration of the original hybrid by crossing DH lines with complementary sets of parental chromosomes.

4.6 Gene stacking from bi-parental/multi parental crosses

In plant breeding, marker-assisted backcrossing is commonly used to introduce one or a specific number of genes into elite inbreds (Leubberstedt and Frei, 2012). A heterozygous plant is selfed to produce a fixed line at the end of backcross programmes while the probability of individuals with the desired homozygous genotype is ¼ for single-gene introgression. According to the formula ¼n, where n is the number of independently segregating genes, the frequency of predicted genotypes decreases exponentially (Leubberstedt and Frei, 2012; Ravi et al., 2010; Shen et al., 2015). Whereas, doubled haploids are generated with a frequency of

½ n. In a four gene introgression programme, the frequency of the required homozygous genotype following traditional breeding process would be 1/256 while the doubled haploid progenies would be 1/16. Thus, the application of doubled haploid technology significantly decreases the population size required to find desirable genotypes (Fig. 7).

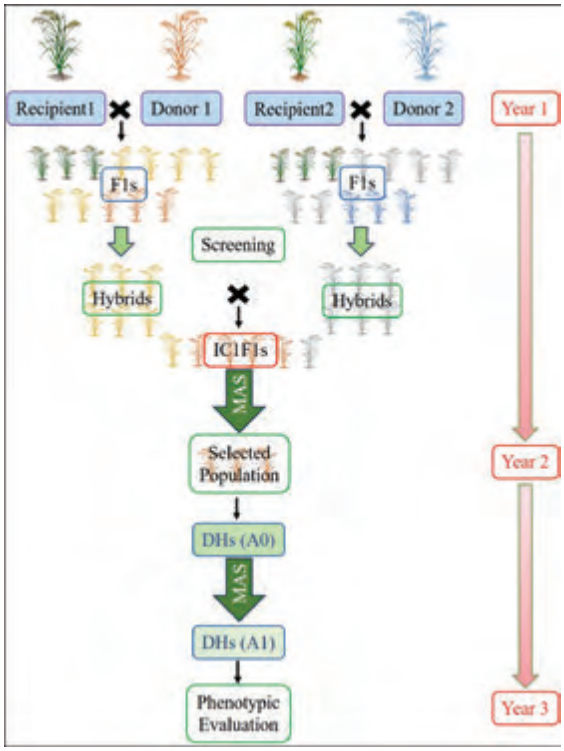

Fig. 7. Timeline for DH assisted pyramiding of multiparental traits

4.7 Accelerating plant breeding by MAS and GS

Breeders use Marker assisted selection (MAS) and Genomic Selection (GS) in crop improvement due to the easy availability of molecular markers. MAS is supported by the observation of markers that are strongly linked to a trait. Breeders may use MAS to eliminate a large number of plants with undesirable gene combinations, pyramid beneficial genes in subsequent generations, reduce field research, and lessen the number of generation (Dwivedi et al., 2015). Combining MAS and DHs opens up new avenues for increasing genetic gain and cutting down on cultivar breeding time. MAS and DHs have been successfully used to accelerate resistance breeding in cereal crops (Wessels and Botes, 2014), demonstrating the

integration of MAS and DH technology to accelerate cultivar growth as compared to traditional breeding processes.

4.8 Transgenic research

The doubled-haploid technique, in general, allows for the rapid homozygous state attainment of heterologous loci. This concept can be applied to transgenic plant to produce homozygous plants in a single generation. The most common method for obtaining homozygous plants for transgenic loci is to self the transgenic plants after regeneration. Anther culture of positive transformed plants in T0 would result in homozygous conditions; in contrast, selfing would result in homozygous conditions only after 3-4 generations of selfing (Fig. 8). Anther culture has been used to successfully produce homozygous transgenic rice with the chitinase gene for improved sheath blight resistance (Baisakh et al., 2001). Using anther culture to produce DHs from transgenics containing genes involved in ß-carotene metabolism could be useful in the future (Datta et al., 2014).

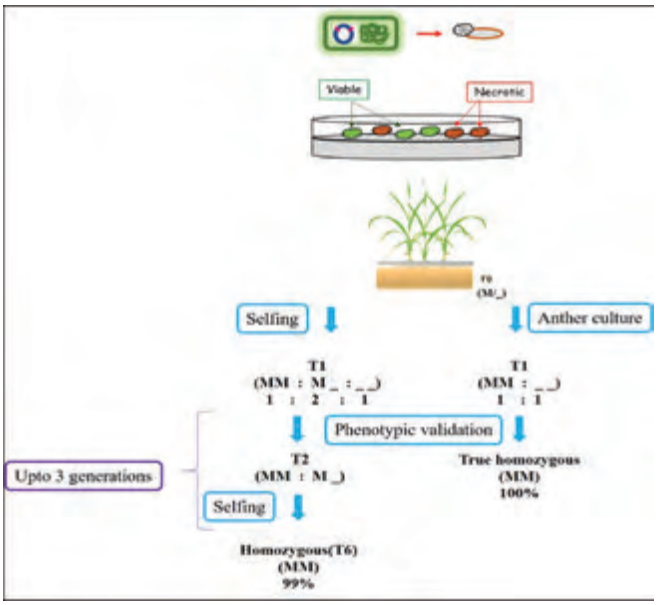

Fig. 8. Development of homozygous mutant lines using DH

5. HAPLOID INDUCER LINES: THE NEXT STEP

The *in vitro* based approach is well developed and relatively competitive in rice haploid and doubled haploid development. While androgenesis has resulted in the production of a number of rice lines, the process is heavily influenced by environmental influences, genotype dependence, and significant somaclonal variation, but it most importantly requires expertise and sophisticated infrastructure. *In vivo* approaches include wide hybridization, intraspecific crosses, which have led to the development of DH production in wheat, maize and may other crop species. In wheat, haploids can be generated by wide hybridization methods especially wheat-maize system (Laurie and Bennett, 1986). Haploid production by intraspecific hybridization is the predominant way even in maize (Chang and Coe, 2009; Geiger, 2009). This method was first reported in 1959 with the discovery of maize haploid inducer, producing 2%–3% maternal haploids

when outcrossed as a male. Over the years, haploid induction rate (HIR) increased to 8%–10% due to development of high inducing lines, such as WS14, RWS, UH400, BHI306 and CAU5 (Wu et al., 2014; Xu et al., 2013). Unlike wheat and maize, rice lacks *in vivo* haploid induction system.

A detailed molecular study reveals the involvement of several genes and QTL (s) in maternal haploid induction, few genes have been cloned and well characterized for haploid induction such as *ZmMATL* (*MATRILINEAL*) in maize (Kelliher et al., 2017; Gilles et al., 2017), *CENH3* gene in Arabidopsis, maize and barley (Ravi and Chan, 2010; Kelliher et al., 2016; Kalinowska et al., 2019), *HFD* gene in *Aarbidopsis* (Kuppu et al., 2015), *CENH3* gene in rice (WO 2017 200386). A rice orthologue of maize *MATL* was recently discovered to be functionally conserved similar to maize *MATL* (Kelliheret al., 2017). In rice, the knockout mutation in *MATL* orthologue (*OsMATL*) resulted in a 2-6 % haploid induction rate (Yao et al., 2018) similar to original *Stock6* of maize. These findings show that *OsMATL* is a promising candidate for developing haploid inducer stocks in rice (Fig. 9), with enormous potential for rice improvement.

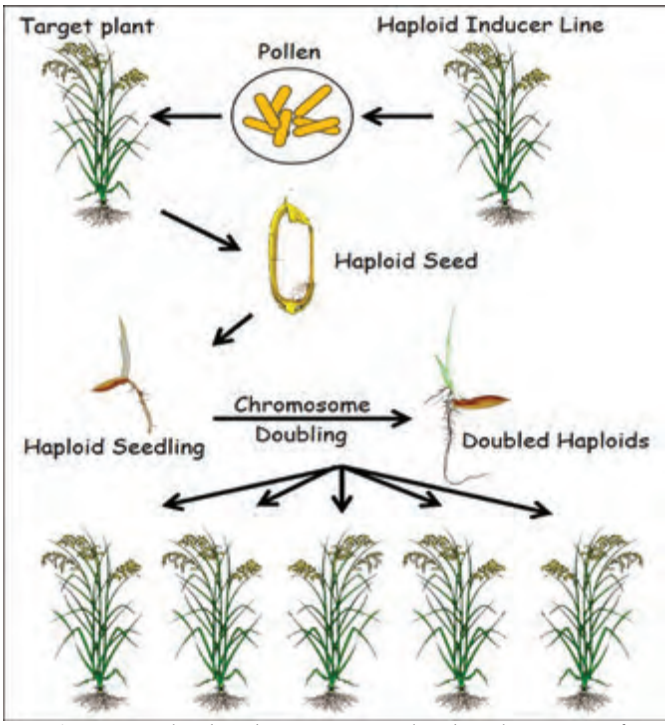

Fig. 9. Haploid Inducer Line in the development of DHs

A *CENH3* mutant of *Arabidopsis thaliana* was crossed to *WT* (*Wild Type*), Ravi and Chan (2010) discovered that it can produce haploids. The endogenous *CENH3* N-terminal tail was replaced with the *green fluorescent protein* (*GFP*) fused N-terminal tail domain of *H3.3* to create this mutated *CENH3* (histone protein tail-swap). Afterwards, Maheshwari et al. (2015) showed that natural variation in *CENH3*of *Brassica rapa* can be exploited to develop HI line. When expressed in the egg cell, the *BBM1* (*babyboom 1*) gene in rice has been shown to cause parthenogenesis and the growth of haploid plants at a high frequency (Khanday et al., 2019). Another maize orthologue *ZmDMP* was found to be effective in the development of HI lines in *Arabidopsis thaliana* with an average haploid induction

rate of 2.1 ± 1.1%, suggesting the extension of the HI system from maize to other crops (Zhong et al., 2020). All of these methods point to the possibility of developing a suitable DH production technology in rice.

New HILs can be created by introducing a targeted mutation in the related genes, which necessitates the use of a precise gene editing tool. Previous approaches were based on the use of TILLING (Targeting Induced Local Lesions in Genomes) which resulted in the development of some off targeted mutations, hampering the HI development in *Arabidopsis thaliana* and rice. Therefore, the availability of advanced and precise tool like CRISPR-cas9 will help grow HIL in rice while also assisting in the improvement of breeding strategies.

6. CONCLUSION

Due to the obvious recalcitrant nature of *indica* rice, it is essential to optimise the anther culture method in order to achieve its full potential yield of androgenesis technology in indica rice. Despite the efforts of Rout et al. (2016) and Naik et al. (2017) to address these issues, new media manipulation attempts to increase callusing capacity or somatic embryogenesis must be introduced. Furthermore, study of the genetic structure in DHs might be used to identify genomic regions that lead to promising yield in rice from heterotic F1 hybrids. Recent research with Arabidopsis and maize (Wang D et al., 2015) suggests that heterosis can be managed by carefully combining genomic regions from the parents that lead to enhanced yield. Apart from the *in vitro* approaches, the breeder friendly HI system must be introduced in rice varietal development in order to fast forward the breeding programme. Coupling advance gene editing system like CRISPR-Cas9 can guide the novel HI approach in rice.

Significance of DHs in varietal development by the quick fixation of segregating loci is well established, and it should be utilized to its full potentiality for which new media compositions should be developed to increase the androgenic capability in indica rice. Simultaneously, we must concentrate on direct somatic embryogenesis from microspores, since it is the most cost-effective of all the tissue culture pathways. Furthermore, the process of spontaneous chromosome doubling during androgenesis needs urgent attention. Besides which, there is still a lack of conceptual understanding of DHs' superior yield. As a result, the theoretical basis for DHs' superior yield should be thoroughly investigated by generating a large population of DHs.

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Genome editing for rice improvement

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SUMMARY

Genome editing has started showing a remarkable impact on agriculture. Editing a genome of interest relies on creating a targeted DNA double-strand break (DSB). ZFN, TALEN and CRISPR-Cas9 have emerged as the three most efficient tools to create targeted DSBs. In this chapter, brief discussion has been made on the basis of these three tools with particular emphasis on CRISPR-Cas9. The application of genome editing for rice crop improvement has also been reviewed. Brief mention has also been made on the major bottlenecks of applying these technologies for rice improvement.

Keywords: Genome editing, double-strand break, TALEN, CRISPR-Cas9

1. INTRODUCTION

Rice, the most widely cultivated crop worldwide, provides food for half a billion population, serves as an excellent model for functional genomic study. Because of high genetic diversity, genomic synteny with other crops, and diverse environmental adaptability, rice has been considered as a strategic crop for food security (Montano et al., 2014). At least one billion populations suffer from a severe nutritional deficiency in the present-day world, while the agricultural system is worsened by the loss of biodiversity and climate change (Foley et al., 2013). With the global population estimated to reach 9 billion by 2050, there will be an urgent need to improve the agriculture with climate-resilient crops (Tilman et al., 2011). Conventional breeding is the most widely adopted approach for crop improvement, but it is time-consuming and laborious. Mutation breeding has the ability to introduce mutation randomly in the crop genomes, using chemical and physical mutagens (Pacher et al., 2017). Transgenic technology offers the possibility to develop superior crop variety by transferring cis or transgenic beneficial traits. Although GM (Genetically modified) crops hold promise for global food security, their use has become restricted by various biosafety regulations (Prado et al., 2014).

Genome editing (GE), a new breeding technology, performs genomic modification at targeted loci (Chen et al., 2019). SSNs (sequence specific nucleases), such as ZFNs (Zinc finger nucleases) (Kim et al., 1996), TALENs (Transcription activator-like effector nucleases) (Christian et al., 2010) depends on protein-DNA

interaction for target specificity, where design and development of active nuclease are complicated and an expensive process. Recently discovered CRISPR (Clustered regularly interspaced short palindromic repeats) and Cas9 (CRISPR-associated protein) (Jinek et al., 2012) is completely based on RNA-DNA interaction, provides simplicity and ease to modify any genomic target sites. The CRISPR-Cas9 introduces double-stranded break (DSBs) at the target DNA site, which is ultimately repaired by either non-homologous end-joining (NHEJ) or homology-directed repair (HDR) pathways (Molla and Yang, 2020). The NHEJ is a major error-prone repair pathway, leads to random insertions, deletions at the cut site, causing knock-outs (Bortesi and Fischer, 2015). On the other hand, HDR is much more precise, involves an exchange of intact DNA fragments, leading to gene knock-in or gene replacements (Voytas and Gao, 2014).

The CRISPR-Cas9 is a two-component system consisting of a protein called Cas9 and guide RNA (gRNA). Cas9 protein is an RNA-dependent DNA endonuclease. The gRNA contains a 5′ 20 nucleotides protospacer sequence complementary to target DNA sequences and is necessary to direct Cas9 protein to the target site.

The CRISPR-Cas9 system has allowed crops to be modified in a single generation. Once an editing agent has been segregated out into the next generation, there will be no difference between a gene-edited product and a naturally mutated one. Therefore, the incorporation of genome editing into modern agricultural practice will enhance the rate of crop improvements and improve breeding strategies (Voytas et al., 2014). Rice, being a model crop, has been successfully utilised as a target for genome editing purposes. Small genome size, wide availability, and ease to transform make rice a suitable crop for functional genomic study (Feng et al., 2013). This chapter will briefly discuss the genome editing technologies focused on CRISPR-Cas9 and their potential use for rice trait improvements such as biotic and abiotic stress tolerance, quality, and diverse parameters determining yield.

2. GENOME EDITING

Genome editing is demarcated as an assemblage of modern molecular biology techniques that enable precise and efficient targeted modifications, *viz.* deletion, insertion, and nucleotide substitution at particular genomic loci (Chen and Gao, 2013).

2.1. Genome editing Tools available for engineering rice gene

2.1.1 Zinc Finger Nucleases (ZFNs)

ZFNs (Zinc Finger Nucleases) are the chimerically engineered nucleases with a structural module of Cys_2-His_2based zinc finger (ZF) (Kim et al., 1996). The basic

structure of ZFNs involves two components: (a) A DNA-binding domain of 300–600 zinc-finger repeats and (b) the DNA cleavage domain consisting of Fok1 restriction enzyme (Carroll et al., 2006; Carlson et al., 2012). ZFN activity is dependent on FokI dimerization (Kim et al., 1996). Every Cys2-His2 ZF domain has 30 amino acid residues, binds to a target DNA sequence with an interaction of á-helical portion of protein and major groove of double-helical DNA (Pavletich et al., 1991). Each zinc finger has the ability to bind triplet nucleotide on the target DNA sequence. ZFNs have two monomers ascribed to bind reversely flanking with 5 to 6 bp to the target DNA site (Minczuk et al., 2008). After the DNA binding domains of a pair of ZFN recognize and bind a DNA sequence, Fok1 dimerizes and cleaves the target DNA (Fig. 1). The application of ZFNs is highly dependent on modular design and optimization of zinc fingers against particular target DNA sequences.

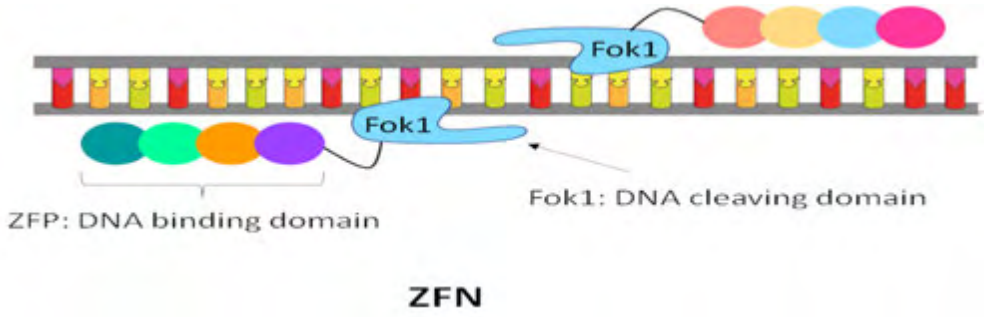

Fig.1 Basic structure and design of a zinc finger nuclease (ZFN) (Adopted from Karmakar et al., 2020).

2.1.2. Transcription Activator-Like Effector Nucleases (TALENs)

TALE proteins, secreted by bacteria, recognize and activate specific plant promoters (e.g., SWEET gene promoter) through a set of tandem repeats, which generates the basis for the creation of a new genome-editing tool called TALE nucleases (TALENs) (Jankele et al., 2014). TALENs are the fusion of the FokI cleavage domain and DNA-binding domains of TALE effector proteins (Zhang et al., 2019). The most widespread use of TALE DNA-binding domains is to create programmable TALENs to generate double stranded break (DSBs) at desired target sites in the eukaryotic genome (Doyle et al., 2013). TALENs function as a pair, as one domain binds explicitly to one DNA strand, while the other binds to the complementary strand, wherein FokI provides DSBs (Fig. 2). TALENs consist of proteins that have the main domain, responsible for DNA binding, and NLS (nuclear localization sequence) (Schornack et al., 2006). Monomers of TALE protein have a central repeat domain (CRD), which allows DNA binding and specificity to a particular host. CRD domain consists of 34 amino acid tandem repeats. Two highly variable amino acids at positions 12 and 13 of the repeat are responsible for nucleotide

recognition. These two amino acid positions are known as Repeat Variable Di-residue (RVD) (Moscou and Bogdanove, 2009). These RVD domains have been successfully used for designing specific DNA binding artificial TALE proteins. Fusion of Fok1 nuclease with TALE DNA binding domain has been established to effectively create a tool for genome modification (Christian et al., 2010). After the recognition and binding of TALEN to a specific DNA sequence, Fok1 dimerizes and cleaves target DNA.

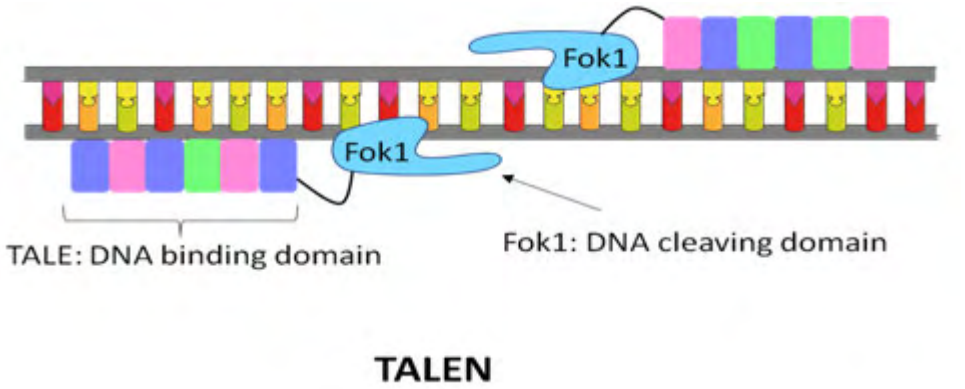

Fig.2 A scheme for introducing double-strand breaks using chimeric TALEN proteins (Adopted from Karmakar et al., 2020)

2.1.3. CRISPR-Cas9

The CRISPR-Cas system is an adaptive defence system found in bacteria and archaeato protect them from foreign DNA and phages (Deveau et al., 2010). It consists of several repeat-interval sequences (CRISPR) and protein, called Cas9, which has both nuclease and helicase activity (Chylinski et al., 2014). To date, six different types of CRISPR-Cas systems have been identified (Makarova et al., 2017). Among them, the most popular one is the type II CRISPR-Cas system identified in *Streptococcus pyogenes*, which consists of two RNAs viz. CRISPR RNA (crRNA) and trans-activating crRNA (tracrRNA), associated with Cas9 protein. Cas9 protein in association with crRNA-tracrRNA forms a complex that can cleave target-DNA sequence if it is located adjacent to the short sequence, called protospacer adjacent motifs (PAM) (Gasiunas et al., 2012).

On the basis of the Cas9 gene, the CRISPR-Cas9 system has been divided into two main classes that are subdivided into six different types. Class 1 CRISPR-Cas9 system (type I, III, and IV) uses multi-Cas protein for DNA cleavage, whereas Class II system (types II, V, and VI) provides targeted interference with single effector protein. This effector protein binds to the targeted DNA with the help of guide RNAs (gRNAs). gRNA has two components *i.e.* crRNA (CRISPR RNA)

and tracrRNA (trans-activating RNA). crRNA binds with tracrRNA to form a single guide RNA (sgRNA). Cas9 protein binds with sgRNA and forms Cas9/sgRNA complex, where 20 nucleotides at the 5′ end of the sgRNA allow this complex to bind at a specific target DNA site. After binding, Cas9 cleaves the target DNA adjacent to 5′-NGG-3′ PAM (protospacer adjacent motifs) (Jinek et al., 2014).

Cas9 protein has two nuclease domains *viz.* HNH and RuvC-like that cleaves complementary/target and non-complementary/non-target DNA strands, respectively (Gasiunas et al., 2012) (Fig. 3). Nickase Cas9 (nCas9) is developed when one of its domains is mutated, whereas mutation to both domains leads to dead Cas9 (dCas9). dCas9 cannot generate cut at the target DNA sites but can bind to a target DNA (Jiang et al., 2017). SpCas9 from *Streptococcus pyogenes*is

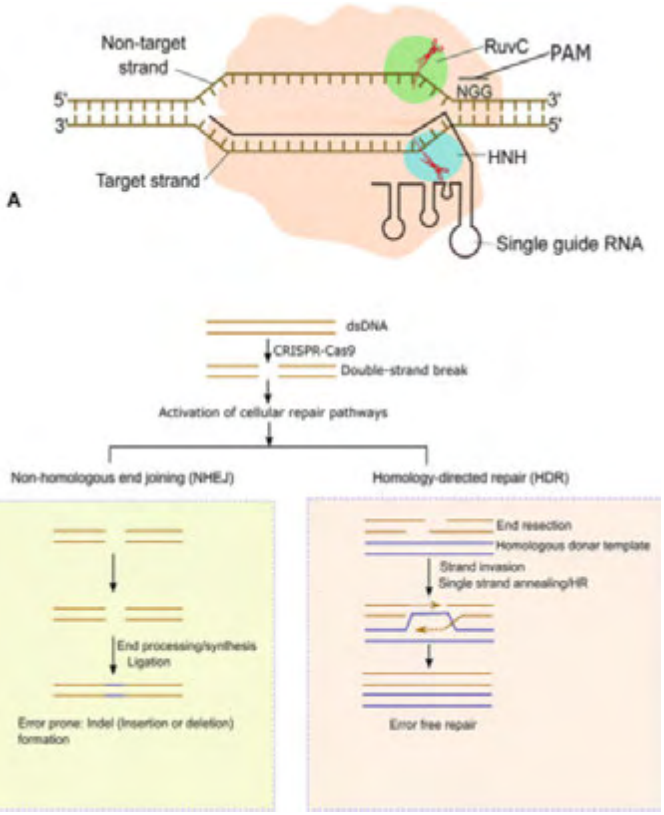

Fig.3 Schematic diagram of CRISPR-Cas9 editing of target genes. *(A) A sketch of CRISPR-Cas9 system. The single guide RNA can identify the target gene, and then the two domains of Cas9 cleave the target sequence. (B) DSB can be repaired through either non-homologous end joining (NHEJ) or homology-directed repair (HDR). (Adopted from Molla and Yang, 2020)*

the most widely used Cas9 that provides DSBs at genomic target sites (Jinek et al., 2012; Cong et al., 2013; Gasiunas et al., 2012). Orthologous Cas9 protein, such as *Staphylococcus aureus* (SaCas9), *Streptococcus thermophilus* (StCas9), and *Neisseria meningitides* (NmCas9) has also been successfully utilized as genome editing tools (Cebrian-Serrano, 2017). Orthologous Cas9 proteins from other bacteria have been enlisted earlier (Molla et al., 2020a). Although many advanced technologies such as base editing, prime editing, CRISPR-mediated gene regulation, and epigenome editing have recently been developed harnessing the targeting power of CRISPR-Cas systems (Molla et al., 2020a), here the discussion has been restricted in this chapter only to conventional knock-out or random indel generation by conventional CRISPR-Cas9 and precise editing through HDR.

2.2. Targeted genomic DSB gives rise to genome editing

ZFN, TALEN, and CRISPR-Cas9 generate targeted DSB in the genome. Once the DNA DSB is made by any of the sequence specific nucleases (SSN), cellular repair machinery comes into action to repair them. Non-homologous end joining (NHEJ) and homology-directed repair (HDR) are the two well-known cellular pathways that exist to repair the DSB (Fig. 3). NHEJ is the predominant one and error-prone. During re-ligating the broken DNA ends, NHEJ often generates random insertion or deletion (Indel). Random indel frequently disrupts the open reading frame, and as a result, the gene function is lost. Hence, DSB generation by nuclease and subsequent error-prone cellular repair knock out gene/s with high efficiency. On the other hand, HDR pathway is utilized for incorporating precise changes in the genome.

3. APPLICATION OF GENOME EDITING IN RICE

3.1. Use of ZFN for rice genome editing

The application of ZFN for targeted genome modification has been reported previously (Martínez-Fortún et al., 2017). The use of ZFN to modify the rice genome was reported by Cantos et al., 2014, where they highlighted the identification of safe regions on the rice genome through ZFN for gene insertion and trait stacking. ZFN mediated disruption of the coding region of the *SSIVa* gene in rice has been reported (Jung et al., 2018). The study helped to elucidate the functional role of this gene. Transgenic rice plants with substitution mutations were generated. Transgenic plants exhibited no expression of *SSIVa* mRNA, low starch contents, and dwarf phenotypes (Jung et al., 2018).

3.2. Use of TALEN for genome editing in rice

The majority of TALEN application on rice is principally based on targeted mutagenesis (Wright et al., 2014). TALEN engineered rice was developed by mutating the *OsSWEET14* promoter region to generate disease-resistant rice lines

(Li et al., 2012). Earlier reports show that TALENs mediated mutagenesis of multiple susceptible genes in rice to develop broad spectrum bacterial blight resistance (Cai et al., 2017; Hutin et al., 2015). Other groups also used TALENs to generate mutations in rice. For example, lipoxygenase *LOX3* was mutated to improve seed storage quality (Ma et al., 2015). *OsBADH2* (betaine aldehyde dehydrogenase 2) knock-out line was developed using TALEN to increase fragrant compound, 2-acetyl-1-pyrroline in rice grain (Shan et al., 2015).

3.3 Significant achievement of CRISPR-Cas9 genome editing technology in rice improvement

Type II CRISPR-Cas9 system from *Streptococcus pyogenes*, has been established as a versatile tool for targeted genomic modification and applied for a wide variety of crop species (Hsu et al., 2014). CRISPR-Cas system, as compared to ZFNs and TALENs, has been implicated as a simple, efficient, and low-cost genome editing tool that can target multiple genes at a time (Cong et al., 2013; Mali et al., 2013). CRISPR-Cas9 has been rapidly adopted in plant systems (Nekrasov et al., 2013; Shan et al., 2013) and can be implemented as an effective solution in plant breeding (Zhang et al., 2018). The most significant achievements of CRISPR-Cas9 has been the null alleles production through gene knock-outs, which implies installation of indels (insertion and deletions) that leads to frameshift of the reading frame (Zhang et al., 2018). Few significant reports for different applications are discussed here.

3.3.1. Abiotic stress tolerance

Abiotic stresses, such as drought, cold, and salinity lead to yield loss and decreased crop productivity. Therefore, deployment of abiotic stress-resilient crops is necessary to safeguard global food security at present under the climate change scenarios. Several approaches have been implemented to understand the regulatory network and pathways leading to stress management. Through conventional breeding/ biotechnological processes, some of the components/genes have been taken for engineering crop plants, which resulted in yield enhancement (Mickelbart et al., 2015). CRISPR-Cas system has been used to delineate the underlying mechanism of stress resistance in crop plants. This system is suitable for generating superior germplasm by deleting some negative regulators or inserting gain-of-function mutation through precise genome editing (Zhu et al., 2020).

CRISPR-Cas techniques have been applied to enhance abiotic stress tolerance in rice by targeting several genes (Bhowmik et al., 2019). CRISPR-Cas9 mediated knocking out of *OsPYL* gene in rice leads to high-temperature tolerance (Miao et al., 2018). In a previous study, the promoter of *OsRAV2* gene was targeted with sgRNA/Cas9, and generated mutant lines develop high sensitivity to salinity (Duan et al., 2016). *OsSAPK2*, an ABA-dependent protein showed elevation in activity during drought stress. Mutant lines, sapk2 was developed through CRISPR-

Cas9 showed ABA- insensitive phenotype, and more sensitivity to drought stress (Lou et al., 2017). It is indicative that CRISPR-Cas9 is contributing significantly to functionally validate abiotic stress responsive genes in rice.

3.3.2. Disease resistance

To increase disease resistance in plants, genome editing holds great promise. Genome editing tools introduce mutations at targeted loci for improving resistance against various biotic stresses. It helps to rapidly develop pathogen-resistant transgene-free crops (Borel et al., 2017). There are diverse approaches to create transgene-free biotic stress-tolerant plant through CRISPR-Cas9, such as (a) A simple knock-out of susceptibility factor encoding genes, (b) deletion or insertion of base sequences at the promoter region of susceptible genes, (c) introduction of specific mutation at the coding region of the gene *via* HDR, (d) changing specific amino acid sequences in a protein, targeted by some pathogen effectors (Schenke et al., 2020). Rice blast is a highly infectious disease, affecting sustainable rice production worldwide. Various studies have been performed to develop high-yielding, disease-resistant rice varieties (Yan et al., 2017). Blast-resistant rice was produced in the Kuiku131 rice variety background by the CRISPR-Cas-mediated knock-out of *OsERF922*gene (Liu et al., 2012). Genome editing of *OsSEC3A*was successfully performed in rice for enhancing resistance against *M. Oryzae* (Ma et al., 2018). Broad-spectrum resistance against bacterial blight pathogens was achieved by mutating three rice susceptible gene promoters, namely SWEET11, SWEET13 and SWEET14 (Xu et al., 2019; Oliva et al., 2019). Rice tungro disease is a significant viral disease of rice, especially in Asia. The factor imparting resistance to particular rice variety depends on a gene called *translation initiation factor 4 gamma (eIF4G)* (Lee et al., 2010). An earlier report described CRISPR-Cas9 mediated genome editing of the gene to develop RTD resistance rice variety (Macovei et al., 2018). Genome editing strategies to develop virus-resistant plants have recently been reviewed (Mustaq et al., 2020).

3.3.3. Increasing yield

Rice yield has been significantly enhanced by knocking out negative regulator genes that hamper grain size, number, and weight (Zhang et al., 2017). Among the various factors governing the yield phenotype, manipulating the cytokinin pathway can be a suitable approach for enhancing yield. In a previous report, genome-edited rice plants, with a modified C terminus of a cytokinin activation enzyme *OsLOGL5,* showed enhancement in yield in diverse environmental conditions (Wang et al., 2020). By knocking out the gene, amino acid permease 3, that involves in nutrient homeostasis, increment in tiller number and yield was achieved (Lu et al., 2018). Editing has been successfully performed in four regulator genes, namely, *Gn1a, DEP1, GS3*, and *IPA1*, using the CRISPR-Cas9 system,

resulting in expected phenotypes (Li et al., 2016). Multiplex editing was performed targeting three-grain weight related genes, namely, *GW2, GW5*, and *TGW6* using CRISPR-Cas9, resulting in a 29.3% increment in 1000-grain weight (Xu et al., 2016).

3.3.4. Improving quality

Agricultural production other than yield for any crop is also very crucial. The CRISPR-Cas9 technique successfully created high amylose rice by targeting two starch branching enzymes *SBEI* and *SBEIIb* (Sun et al., 2017). A previous study highlighted the creation of loss of function mutation through CRISPR-Cas9, targeting the first exon of the *Waxy* gene (Zhang et al., 2018).

Heavy metals in food impart serious health problems. Eradication of heavy metals from soil helps to reduce their accumulation in rice grain. Genome editing has been implicated in the reduction of accumulation of toxic metals in the edible part of the food. Cadmium is carcinogenic and toxic for human health (Clemens et al., 2013). Rice, a food crop, a major source of caloric intake, contains excessive cadmium (Cd). CRISPR-Cas9 has been used to generate low-Cd rice through knocking out of transporter gene, *OsNramp5*. Mutated rice lines showed a significant reduction in the level of Cd without affecting any detrimental effect in phenotype (Tang et al., 2017).

The Bio-fortification of â-carotenes is an essential target for improving rice quality. The *Osor* gene was targeted in rice using CRISPR-Cas9. The site-directed modification of *Osor* in rice through CRISPR-Cas9 resulted in enhanced β-carotene accumulation in rice callus (Endo A et al., 2019).

3.3.5. Herbicide resistance

Herbicides, namely, chlorsulfuron and bispyribac-sodium (BS) are applied in the rice fields to control weeds. These herbicides target the enzyme acetolactate synthase (*OsALS*) (Zhou et al., 2007), resulting in inactivation of enzyme and subsequent impairment in the biosynthesis of branched chain amino acids. Genome editing tool CRISPR-Cas9-mediated HDR was used to introduce targeted point mutations in *OsALS* (Sun et al., 2016). Genome-edited plants were survived and grew normally after spraying with herbicide bispyribac sodium, whereas wild-type plants survived for few days. Similar to the earlier report, herbicide resistance rice was also developed by nucleotide substitution of the rice gene, *5-enolpyruvylshikimate-3- phosphate synthase* (*EPSPS*) using CRISPR-Cas9. The engineered version of *EPSPS* had two amino acid substitutions *i.e.*, T102I and P106S, conferring resistance to the glyphosate (Li J et al., 2016). Another report demonstrated editing of rice *Bentazon Sensitive Lethal* gene (BEL) for developing herbicide resistance (Xu et al., 2014). Similarly, other genes that are targeted by herbicides can be edited through HDR to make them insensitive to herbicides.

4. CONCLUSION AND FUTURE PROSPECT

CRISPR-Cas9 has emerged as a revolutionary tool in plant science research. It is truly a game-changing tool for crop improvements that create mutations with a high level of precision and accuracy within genomic target loci. A major advantage of this technology is that the transgene causing modifications can be easily eliminated from the genome in the subsequent generation, leaving only mutated plants. In addition to the targeted knock-out induced by CRISPR-Cas9, several other editors like base editors, prime editors, and epigenome editors have been developed (although not considered in this chapter), which can perform precise genome modification. With the help of these novel tools, a large number of crop varieties can be developed with superior agronomic characters (Zhu et al., 2020).

CRISPR-Cas12a has also emerged as another powerful genome-editing tool for crop improvements. CRISPR-Cas12a system is also suitable for performing robust multiplex editing (Chen et al., 2018). Genome editing-based epigenetic regulations also hold promise in crop improvements, as mutation can be inherited to off springs without any change in the genomic sequence. Such epigenome editing tools and their development in plant systems will open a new avenue to the genome-editing-based improvements in rice and other crops. Precise editing through HDR is inefficient in plants. Base editing is another remarkable development in precise genome editing that can install point mutations without inducing genomic DSB and is more efficient than HDR (Molla and Yang, 2019; Molla et al., 2020b). However, these versatile tools have not met all the needs for plant genome modification. The first challenge is to identify a specific PAM site for Cas9 nuclease. SpCas9 has NGG PAM requirements, has been extensively used for genome editing. Rapid discovery of orthologous Cas9 and efforts in engineering SpCas9 are playing an important role in removing the PAM constraints and thus expanding genome targetability (Molla et al., 2020a). Those Cas9 variants are making their way in genome editing applications in plants. These recent findings will help expand the scope of genome editing in rice, using orthologs and engineered version of Cas9 with altered PAM requirements. Second challenge is that some valuable traits in plants controlled by quantitative trait loci and CRISPR-Cas mediated genome editing tools cannot perform sufficient change to install all modifications necessary. There will be a need to develop more advanced genome editing tools for stacking multiple mutated alleles (Zhu et al., 2020).

Third challenge is that CRISPR-Cas-mediated editing can sometimes generate off-target editing, although at an extremely low rate, that might negatively impact plants. Cas9 variants are being engineered for higher specificity to reduce off-target editing. Another critical factor in achieving a high success rate in genome editing is to have a reliable transformation and regeneration system. All rice

genotypes are not equally responsive to tissue culture. Delivery of CRISPR-Cas reagents in plant systems is still problematic and challenging. Therefore, developing new gene delivery methods, such as nano particles (Demirer et al., 2019), DNA nano structures (Zhang et al., 2019), virus-mediated or cell-penetrating peptides (Santana et al., 2020) are promising for efficient delivery of CRISPR-Cas reagents in plant system, including rice. More research is required in above-discussed areas to realize a higher impact of genome editing in a wide range of rice genotypes.

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Genomics and other omics approaches for rice improvement

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SUMMARY

Rice (*Oryza sativa* L.) is considered as the model crop for different omics studies in monocotyledon on species. A thorough understanding of the regulatory mechanisms of different traits related to climate resilience, higher yield, input use efficiency, grain and straw qualities, value addition, etc in rice is critical for global food security. During the last 30 years, several advanced omics platforms and bioinformatics tools have been developed and utilized, which further facilitated for identification and cloning of several QTL/genes and understand their biological function. These developments helped the cloning of over 3000 genes controlling different traits in rice. Some of them have been characterized in detail/partially for their biological functions at the molecular level. Some rice varieties have been developed and commercialized in different countries by pyramiding of few major QTL/ genes. These varieties were found to have better climate resilience, higher yield, better grain qualities, nutrient use efficiency, etc. In this chapter, we briefly review the advances in rice omics research during the past 30 years. These achievements made in omics, such as genomics, transcriptomics, proteomics, micromics, and metabolomics research will greatly facilitate our understanding of gene regulation of different traits, and utilize them in translational breeding programs. We also discuss future prospects and trust areas of rice omics research.

Keywords: omics, monocotyledon, pyramiding, QTL/genes, proteomics, transcriptomics, genomics

1. INTRODUCTION

Rice provides dietary energy for more than 3.5 billion people. It is cultivated under diverse ecosystems. In the last four decades, rice yield has been doubled especially during the 1960s to 2000s, primarily as the result of genetic improvements of existing rice varieties, such as the introduction of the semi-dwarf varieties and hybrids. Still, we have to increase rice yield significantly in order to meet food demand for the ever-growing human population and for safeguarding food security (Mohapatra et al., 2021). Rice is considered as a model crop for omics research due to its small size genome, availability of the high-quality whole-genome sequences of both *indica* and *japonica* subspecies, and also wild rice, efficient transformation technology, co-linearity of sequences with other cereal crops, and

abundant genomic and germplasm resources (He et al., 2018; Xiong et al., 2020). Various omics platforms and resources have been developed, especially during the last 20 years, such as Next-generation sequencing technologies, microarray, generation of mutant libraries, full-length cDNA libraries, gene expression microarrays, and bioinformatics tools, etc (Jiang et al., 2012; Ram et al., 2020). To support omic research, high throughput reliable, multifunctional, phenomic facilities have also been established (Zhao et al., 2019a). The higher-performing platforms for bioinformatics analyses and different databases with higher data capacity have been developed in rice (Rajasundaram and Selbig, 2016). Our knowledge on QTL/genes and their interactions as a whole system approach will improve with newly gained knowledge from omics research. Different omics approaches such as genomics, transcritptomics, proteomics, micromics, and metabolomics works in a holistic manner to reduce knowledge gap between phenotype and genotype through re-sequencing of rice germplasm, mapping and cloning of QTL/genes associated with different traits, understanding of gene functions, and their interactions, allele mining for useful traits, and finally utilize them in translational breeding programs. In this chapter, we summarized our discussion into 7 aspects: (1) whole-genome sequencing of rice germplasm; (2) genomics tools for identification and cloning of QTL/genes for biotic and abiotic stresses, and yield-related traits; (3) Gene prospecting and allele mining; (4) role of transcriptomics in biotic and abiotic stresses; (5) role of micromics in biotic and abiotic stresses, and yield related-traits; (6) role of proteomics in biotic and abiotic stresses, and yield-related traits and (7) role of metabolomics for biotic and abiotic stresses.

2. WHOLE- GENOME SEQUENCING OF RICE GERMPLASM

Whole-genome sequencing is a major step towards revealing genomic diversity, and its efficient utilization in crop improvement. The availability of the complete rice genome sequence (Goff et al., 2002; Yu et al., 2002), the advancement of next-generation sequencing (NGS) technologies, and bioinformatics tools have provided an opportunity for the whole genome-sequencing of several rice genotypes, and the discovery of genome-wide DNA variations such as SNPs, InDels, structural variations, and mining of diversity of genes and alleles in relatively low-cost, and high-throughput manner (Gao et al., 2012). Several cultivated rice genotypes, weed rice, and wild species have been re-sequenced using NGS technology, and millions of DNA polymorphisms have been identified. These variants have been used for identification QTL/genes associated with different phenotypic traits, positional cloning, analysis of haplotypes/allelic diversity, identification of seed purity of varieties, differentiation and diversity between different cultivars, the genetic basis of low glycemic index, insights into breeding history, genome compositions and phylogeny. Several studies reported

the whole genome re-sequencing of rice genotypes and identification of millions of DNA polymorphisms. The rice genotypes re-sequenced including high-quality *japonica* variety Koshikari, Omachi (a Japaneese landrace used for sake brewing), restorer lines (IR24, MH63, and SH27), elite *indica* rice inbred lines (three CMS and three R lines), Korean rice varieties (Hwayeong and Dongjin), maintainer line (V20B), Tongil (a semi-dwarf high-yielding rice variety), Kavuni (a traditional rice cultivar with nutritional and therapeutic properties), and salt-tolerant rice cultivar SR86 (Yamamoto et al., 2010; Arai-Kichise et al., 2011; Li et al., 2012; Subbaiyan et al., 2012; Jeong et al., 2013; Hu et al., 2014, Rathinasabapathi et al., 2016; Chen et al., 2017a). Li et al. (2014) re-sequenced 3000 rice accessions collected from 89 countries and identified 18.9 million SNPs. It is a major step towards revealing the genomic diversity in all of the world's rice germplasm collections. Rathinasabapathi et al. (2015) re-sequenced Swarna, which helped to understand genetic basis of low glycemic index. Yamamoto et al. (2018) performed whole-genome re-sequencing of four NERICA lines, their parents, *Oryza sativa* WAB56-104, and *Oryza glaberrima* CG14. Introgression of *Oryza glaberrima* segments revealed potential causal genes for some agronomic traits including grain shattering, awnness, susceptibility to bacterial leaf blight, and salt tolerance. Lachagari et al. (2019) re-sequenced purple-colored landrace rice cultivar and identified unique allelic variations involved in anthocyanin and flavonoid pathways. Islam et al. (2020) re-sequenced black hulled (PSRR-1) and straw hulled (BHA1115) weedy rice along with *japonica* rice cultivar, Cypres. Haplotype analysis of selected genes involved in domestication, adaptation, and agronomic performance supported the evolution of weedy rice through de-domestication. They also validated the function of several genes such as *qAn-1, qAn-2, Bh4, Rc, SD1, OsLG1*, and *OsC1*. Several candidate genes validated which were identified in previously reported seed dormancy and seed shattering mapping studies. Kishor et al. (2020) carried out whole-genome sequencing of 60 rice varieties belonging to *indica*, *aus*, aromatic, temperate *japonica*, and tropical *japonica* groups. Genetic diversity and comparative analysis based on genome sequence variation revealed that the Basmati genome was derived mostly from the *aus* and *japonica* groups. Tanaka et al. (2020) performed whole-genome re-sequencing of 69 accessions of NARO World Rice Core Collection (WRC) and examined their sequence variation and genetic diversity. They identified a total of 2,805,329 SNPs and 357,639 INDELs. Cultivated rice, *O. glaberrima* and wild species, namely, *O. rufipogon*, *O. nivara*, *O. barthii*, *O. glumaepatula*, *O. meridionalis*, *O. longistaminata*, *O. punctata*, *O. brachyantha* and *Oryza rufipogon* have been sequenced and millions of variations have been identified (Huang et al., 2012; Xu et al., 2012; Chen et al., 2013; Jacquemin et al., 2013; Wang et al., 2014; Li et al., 2020a).

3. GENOMICS TOOLS FOR IDENTIFICATION AND CLONING OF QTL/ GENES FOR BIOTIC AND ABIOTIC STRESSES, AND YIELD RELATED TRAITS

Initially, low throughput marker techniques such as RFLP, RAPD, AFLP, SSR, etc. were used to identify QTL/genes for different traits of rice. With the development of high-throughput genotyping platform such as SNP genotyping system, next-generation sequencing technology, bioinformatics tools, etc., the pace of identification of QTL/genes has greatly enhanced. Several QTL/ genes were identified for biotic and abiotic stresses, yield-related traits, grain quality traits, nutrient use efficiency, etc. using different mapping populations like F_2, RILs, DHs, BILs, RILs, NILs, MAGIC, and association panels. Further, high throughput bioinformatics analysis tools and platforms helped to analyze whole-genome sequence, gene sequence, association analysis, and to develop functional markers. Many useful QTL/genes have been cloned, characterized at the molecular level for their biological functions. Few QTL/genes, especially for biotic and abiotic stresses, grain yield-related traits, and quality traits have been transferred into many elite rice cultivars with higher resilience, grain-yield, and quality, and nutrient use efficiency. These rice cultivars have been cultivated in many countries.

3.1 Identification and cloning of QTL/ genes for resistance to insect pests

Insect pests are serious constraints to rice production. Yield loss due to insects has been estimated at about 25% in Asia alone (Savary et al., 2000). Insects not only damage the plant by feeding on its tissue but also are vectors of devastating rice viruses in many cases. All portions of the plant, from panicle to root, are possibly attacked by various insects. All growth stages of the rice plant, from the seedling to mature stages, are vulnerable. Even after harvest, the grains in store face the insect attacks (Cramer et al., 1967). Because of the limited resistance sources in cultivated rice, it is important to keep exploring resistant germplasm from diverse rice species for cultivar improvement. Many QTL/genes have been identified for major insects like brown planthopper, white-backed planthopper, gall midge, green rice leafhopper (*Nephotettix cincticeps*), and leaf folder. Few resistance genes have been cloned, especially for resistance to BPH.

3.1.1 Brown plant hopper

Brown planthopper (*Nilaparvata lugens* Stal) is a major and serious insect pest of rice in Asian countries, causing huge yield loss. The development and use of BPH-resistant varieties with different mechanisms of host plant resistance through molecular breeding approaches are extremely important to control this pest. With the use of different genomics tools, it has become possible to identify thirty-eight major genes and several QTL resistance to BPH from cultivated and wild germplasm of rice (Jena and Kim, 2010; Hu et al., 2016a; Balachiranjeevi et al.,

2019; Du et al., 2020). Twenty genes (*Bph1, bph2, Bph3, bph4, Bph6, bph7 , Bph9, Bph16, Bph17, bph19(t), bph25, Bph26, Bph27(t), Bph28(t), Bph 30, BPH31, Bph32, Bph33, Bph37*, and *BPH38(t))* have been identified using cultivated rice genotypes (Balachiranjeevi et al., 2019; Li et al., 2019a; Yang et al., 2019a; Du et al., 2020, Yuexiong et al., 2020). Similarly, eighteen resistance genes (*Bph6, Bph10, bph11(t), Bph12, Bph13, Bph14, Bph15, Bph18, Bph20(t), Bph21(t), bph23(t), Bph24(t), Bph27, BPH29, Bph34, Bph3*5 and *Bph3*6) have been identified from seven wild species (*O. australiensis, O. officinalis, O. latifolia, O. eichingeri, O. minuta, O. rufipogon* and *O. nivara)*. Several QTL associated with BPH resistance have been mapped (Mohanty et al., 2017; Yang et al., 2019). The major genes, *Bph1, bph2, Bph3, Bph14, Bph15, Bph18, Bph31,* and QTL *qBph3* and *qBph4* have been widely used for introgression into elite rice cultivars through marker-assisted breeding approach (Sharma et al., 2004a; Jairin et al., 2009; Hu et al., 2015; 2016b). *Bph6* and *Bph30* exhibited strong resistance to BPH showing significant association with four different phenotypic parameters, namely, honeydew excretion, nymphal survival, percent damage, and feeding mark. Eight genes (*Bph3, Bph6, Bph9, Bph14, Bph18, Bph26, bph29,* and *Bph32*) have been cloned and their mode of actions studied at the molecular level (Du et al., 2020). These are found to encode coiled-coil, nucleotide-binding, and leucine-rich repeat (CC-NB-LRR) protein, which is involved in the activation of salicylic acid (SA) and jasmonic acid (JA) pathways, and mediate sucking inhibition in the phloem sieve element or primarily expressed in vascular bundles, activates salicylic acid (SA) signaling and induces callose deposition on rice sieve tubes, impairing feeding of BPH.

3.1.2 White-backed planthopper

White-backed planthopper (WBPH, *Sogatella furcifera*) is a serious insect pest causing severe yield loss to rice in Southeast Asian countries. The development and use of WBPH resistant cultivars is the best alternative to control this pest. In order to improve resistance in susceptible varieties by accumulating favorable genes/QTL with different mechanisms, attempts have been made to identify QTL/genes for WBPH resistance. Twelve resistance genes such as *Wbph1, Wbph2, Wbph3, wbph4, Wbph5, Wbph6(t), Wbph7(t), Wbph8(t), wbph9(t), wbph10(t), wbph11(t*) and *Wbph12(t)* have identified using resistant cultivars, Nagina 22, ARC10239, ADR52, Podiwi A8, N'Diang Marie, Guiyigu, B5 (*O. officinalis*) and Sinna Sivappu, respectively (Du et al., 2020). Similarly, many QTL have been identified (Fan et al., 2018a; Du et al., 2020; Kim et al., 2021). Only few of them have been used in molecular breeding programs. *In silico* analysis of some major genes and QTL led to the identification of candidate genes, which belong to kinase, phytohormones, transcription factors, flavanone 3-hydroxylase (F3H), and especially secondary metabolites (Kim et al., 2021). However, no WBPH resistance genes have been successfully cloned.

3.1.3 Gall midge

The Asian rice gall midge (*Orseolia oryzae* Wood-Mason) is a major insect pest of irrigated and shallow water rice ecosystems in Asia and Southeast countries. Many resistant varieties have been developed utilizing the resistant donors of gall midge and cultivated extensively. The maggots feed on the growing tip, causing the formation of a tubular leaf sheath, called 'silver shoot', in place of inflorescence. To date, eleven midge resistance genes, *Gm1, Gm2, gm3, Gm4, Gm5, Gm6, Gm7, Gm8, Gm9, Gm10,* and *Gm11* have been identified using resistant rice cultivars, W1263, Phalguna, RP2068-18-3-5, Abhaya, ARC5984, Duokang 1, RP2333–156-8, Aganni, Line9, BG 380–2 and CR57-MR1523, respectively (Katiyar et al., 2001; Biradhar et al., 2004; Himabindu et al., 2010; Nanda et al., 2010). *Gm1* imparts resistance to biotypes 1, 3, 5, and 6, while *Gm2* shows resistance to biotype 1, 2, and 5. The recessive gene *gm3*i exhibits resistance to biotype 1, 2, 3, 4, and 7, while *Gm4* imparts resistance to biotypes 1, 2, 3, and 4. Seven dominant genes, *Gm1, Gm2, Gm5, Gm6, Gm7, Gm9* and *Gm10* found to lack resistance against biotype 4. The putative candidate genes for *gm3*, *Gm4,* and *Gm8* are found to encode NB-ARC protein, leucine-rich repeat (LRR) protein and proline-rich protein, respectively (Mohapatra et al., 2014; Sama et al., 2014; Divya et al., 2015, 2018).

3.1.4 Green leafhopper and Green rice leafhoppers

Green leafhopper (GLH) and green rice leafhopper (GRH) are species of leafhoppers and are common pests of cultivated rice in Asia (Angeles and Khush 2000). Fourteen major genes, *GLH1, GLH2, GLH3, glh4, GLH5, GLH6, GLH7, glh8, GLH9, glh10, GLH11, GLH12, GLH13,* and *GLH14* have been identified for resistance to green leafhopper using rice cultivars, Pankahari 203, ASD7, IR8, PTB8, ASD8, TAPL 796, Maddani Karuppan, DV85, IR28, IR36, IR20965–11–3-3, ARC10313, Asmaita, and ARC11554, respectively (Du et al., 2020). Seven major genes, *GRH1, GRH2, GRH3, GRH4, GRH5, GRH6*, and *qGRH7* have been identified for green rice leafhopper resistance using rice cultivars, IR24, DV85, Rantaj emas 2, DV85, W1962 (*O. rufipogon*), SML17, and IRGC104038 (*O. glaberrima*), respectively (Du et al., 2020). No gene has been cloned yet.

3.1.5 Leaf folder

Rice leaf folder (*Cnaphalocrocis medinalis*, Guenée) is a leaf-feeding insect and becoming major pest in the major rice-growing regions of many Asian countries like Japan, India, China, Sri Lanka, Vietnam, Korea and Malaysia due to the heavy use of insecticides and fertilizers (Sun et al., 2013). Leaf folder larvae make rice leaves like hollow cylinder longitudinally and stitch the leaf edge and chew inside the fold by scraping the green matter. The leaves become membranous and turn whitish. In case of heavy infestation, photosynthesis, general vigour, and

plant growth are drastically reduced. Further, the infected plants are then predisposed to bacterial and fungal infections like sheath rot, and faces difficulty in heading, causing severe yield loss at the reproductive stage (Xiao 1990; Rothschild 1996; Padmavathi et al., 2013). Few QTL (*qRLF1, qRLF2, qRLF3,* and *qRLF8*) resistance to leaf folder has been identified using doubled haploid population of CJ06/TN1 and Chuanjiang 06/ TN1 (Rao et al., 2010; Du et al., 2020).

3.2 Identification and cloning of QTL/ genes for resistance to diseases

Rice diseases such as bacterial blight, blast, sheath blight, and false smut are major obstacles for achieving higher rice production. Molecular breeding and transgenic approaches are increasingly being integrated with conventional breeding methods for genetic improvement of disease resistance, and reduction of pesticide usage. During the past two decades, a wide variety of QTL/genes and mechanisms involved in rice defense response have been identiûed and elucidated. However, most of the cloned genes confer a high level of race-specific resistance and works as per gene-for-gene theory. The resistance is effective for only a few years because the pathogen race or strain keeps changing for survival in nature. Therefore, there is an urgent need to broaden the rice gene pool from diverse resources is an ideal option.

3.2.1 Bacterial leaf blight

Rice bacterial blight (BB) caused by bacteria, *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most devastating diseases in tropical and subtropical regions. BB is controlled by both major genes (qualitative) and minor genes (quantitative). The development of rice varieties by pyramiding of major resistance genes/QTL is one of the effective and economic strategies to control this disease. To date, 46 major genes (*Xa1* to *xa46t*) conferring resistance to various strains of *Xoo* have been identified using cultivated, wild rice, and mutants lines (Kim 2018; Kim and Reinke, 2019; Chen et al., 2020; Pradhan et al., 2020). Eighteen genes, namely, *xa5, xa8, xa13, xa15, xa19, xa20, xa24(t), xa25, xa26(t), xa28(t), xa31(t), xa32(t), xa33(t), xa34, xa41(t), xa42, xa 44 (t)* and *xa46(t)* are recessive, while rest 28 genes are dominant (Chen et al., 2020; Neelam et al., 2020). A total of twelve genes have been cloned, of which eight (*Xa1, Xa3/Xa26, Xa4, Xa7, Xa10, Xa21, Xa23,* and *Xa27*) are dominant, while four genes are (*xa5, xa13, xa25*, and *xa41t*) recessive (Kim et al., 2015; Zhao and Zhang et al., 2021). These resistance genes mostly encode nucleotide-binding site–leucine-rich repeat (NBS-LRR) and the cell surface pattern recognition receptors. The recessive genes, *xa13, xa25* and *xa41* belong to the SWEET multiple genes. Xu et al. (2019) edited simultaneously EBEs in the promoters of these three SWEET genes, *OsSWEET11* (*xa13*), *OsSWEET13* (*xa25*), and *OsSWEET14* (*xa41*) using CRISPR/Cas9 technology, and the edited lines

showed broad-spectrum resistance to *Xoo*. Resistance genes *Xa21*, *xa13*, *xa5* and its combinations are widely deployed for resistance breeding through gene pyramiding approach (Pradhan et al., 2015; Pradhan et al., 2016a). IR24 NILs (IRBB) containing genes *Xa4, xa5, Xa7, xa13, Xa21,* and their combinations have been developed by IRRI, Philippines, and used extensively to develop resistance varieties in different countries. Besides, these genes, *Xa38* and *Xa39* have also been recently deployed in breeding programs.

3.2.2 Blast

Blast disease caused by the fungus, *Magnaporthe oryzae*, is a serious disease causing reduction of rice yield up to 10-35% in most rice-growing areas in the world. To date, more than 100 major genes and over 500 QTL have been identified for resistance to blast disease (Ashkani et al., 2015; Li et al., 2019b). However, only twenty-five QTL/genes (*Pi-sh, Pi-t, Pi-37, Pi-b, pi-21, Pi-63, Pi-z, Pi-2, Pi-9, Piz-t, Pi-d2, Pi-d3, Pi-25, Pi-36, Pi5/Pi3/Pi-I, pi-56, Pi-a, Pi-C039, Pi-k, Pi-1, Pik-h/Pi-54, Pik-m, Pik-p, pb1* and *Pi-ta*) have been cloned and well-characterized at molecular level (Li et al., 2019b). Most of known blast resistance genes encode nucleotide-binding site-leucine-rich repeats (NBS-LRR) proteins, except for *Pid2*, *pi21* and *Ptr*. Blast resistance genes, *Pi9, Pigm, Pi50, pi21, Pi7, Pi57* and *Ptr* confer broad-spectrum blast resistance (Li et al., 2019b). Some of major resistance genes, namely, *Pi1, Pi2, Piz5, P9, Pi33, Pi54, Pita, Pib, Pia,* and *Pi-kh* have been used extensively in MAS breeding programs, and few resistance varieties have been developed and cultivated in different countries (Ning et al., 2020). Pyramiding the appropriate resistance genes can effectively achieve strong, broad-spectrum,and durable resistance to blast. *pi21* + *Pi34* + *qBR4-2* + *qBR12-1* (Fukuoka et al., 2015), *Pi5* + *Pi54* + *Pid3* + *Pigm*, and *Pik* + *Pita* together (Wu et al., 2015a) were separately pyramided, and showed strong and broad-spectrum resistance.

3.2.3 Sheath blight

Sheath blight (ShB) (*Rhizoctonia solani* Kühn) is becoming a major disease of rice, causing yield loss 5.0% to 69%. Most of the rice varieties are susceptible to the ShB disease. Only a limited number of moderate-resistant sources are available. Major resistance genes have not been yet identified in rice varieties. However, QTL with minor and major effects have been identified in various rice germplasm. More than 200 QTL associated with sheath blight resistance have been identified using different mapping populations (Zarbafi and Ham 2019; Molla et al., 2020, Bal et al., 2020). QTL, namely, *qSM9-2,* and *qSB12-2* have been introgressed from Teqing into Lemont through the MAS approach (Wang et al., 2012). Similarly, three ShB resistance QTL (*qSBR11-1TE*, *qSBR11-2TE*, and *qSBR7-1TE*) have been transferred into Pusa 6B, maintainer line of aromatic hybrid rice Pusa RH10,

while *qSBR11-1TE* into Improved Pusa Basmati1 from Tetep through MABC. Candidate genes responsible for rice sheath blight resistance have been identified by transcriptome analysis for some of QTL (Zhao et al., 2008; Bal et al., 2020) and genome sequencing (Silva et al., 2012). No SB-QTL have been cloned yet. Though most of the ShB resistance QTL have been identified, so far only a few QTL have moderate effects on ShB resistance. Due to unavailability of any major QTL/gene governing the resistance, there is a need for accumulating multiple QTL from different backgrounds, which shows positive QTL and QTL interaction (Molla et al., 2020).

3.2.4 False smut

False smut disease not only causes a significant yield loss but also deteriorates grain quality by producing a variety of mycotoxins, such as ustiloxins and ustilaginoidins, which are toxic to both humans and animals (Li et al., 2019c). No rice cultivar has yet been identified to have complete resistance to false smut disease, while some cultivars exhibit moderate levels of field resistance to this pathogen (Li et al., 2008; Zhou et al., 2014). More than 52 QTL showing moderate resistance to false smut have been identified using few rice cultivars (Xu et al., 2002; Zhou et al., 2014; Wang et al., 2019a; Han et al., 2020). Two QTL (*qFsr10* and *qFsr12*) have been identified in Lemont (Xu et al., 2002). Similarly, 7, 10 and 5 QTL have been identified using IR28, Lemont/Teqing introgression lines, and MR183–2, respectively (Zhou et al., 2014; Han et al., 2020).

4. IDENTIFICATION AND CLONING OF QTL/ GENES FOR TOLERANCE TO ABIOTIC STRESSES

Abiotic stresses including drought, submergence, salinity, high and low temperatures are limiting the productivity of rice crop in large areas of the world. According to Hossain (1996), abiotic stresses affect rice cultivation more than biotic stresses. There is, therefore, a strong demand for tolerant rice cultivars to abiotic stresses.

4.1 Drought

Drought is one of the major abiotic stresses for rice production, and more than half of the global rice area suffers from the drought of various intensities. Drought tolerance traits are controlled by multiple QTL. More than 1200 QTL related to morphological, root, physiological, biochemical, and yield-related traits associated with drought tolerance have been identified (Barik et al., 2019; Barik et al., 2020; Samal et al., 2020; Yang et al., 2020; Panda et al., 2021). However, only 18 QTL (*qDTY1.1, qDTY1.2, qDTY1.3, qDTY2.1, qDTY2.2, qDTY2.3, qDTHI2.3, qDTY3.1, qDTY3.2, qDTY4.1, qDTY6.1, qDTY6.2, qDTY9.1, qDTY9.1A, qDTY10.1, qDTY10.2, qDTY12.1*, and *qPDL1.2*) associated with grain yield under the reproductive stage drought stress have been reported, and few of them have been

extensively utilized in molecular breeding programs. Yang et al. (2020) identified 82 candidate genes using meta-QTL analysis. These candidate genes include *bZIP, MYB, DREB, WRKY, NAC, AP2/EREBP, bZIP, OsWR2*; late embryogenesis abundant (*LEA*), *DEEPER ROOTING 1*, abscisic acid-stress-ripening-inducible 5 protein, basic helix loop helix protein, inositol1,3,4-trisphosphate 5/6-kinase gene, myo-inositol oxygenase gene, receptor-like cytoplasmic kinase, abscisic acid-inducible mitogen-activated protein kinase, wax accumulation genes ethylene response factor, *CDPK7, CDPK03, CDPK12*, *Dro1*, etc.

4.2 Submergence

Submergence is a major limiting factor for rice production, especially in areas of high rainfall. The development of rice varieties with QTL/genes tolerance to submergence are essential to provide stable yields in flood-prone rice areas. The major QTL, *SUB1* on chromosome 9 was identified using IR40931-26 derived from the landrace, FR13A. It is the major source for tolerating up to 2 weeks of submergence stress depending on the genetic background and the environmental conditions during flooding. *SUB1* was fine-mapped, FR13A derived line was sequenced, and subsequently cloned as a cluster of three ethylene response factor (ERF) genes, namely, *SUB1A, SUB1B*, and *SUB1C* (Xu et al., 2000, 2006). Further, it was proven that *SUB1A* was the key gene that contributes to submergence tolerance (Septiningsih et al., 2009; Singh et al., 2010). *SUB1A* suppresses the production of ethylene through the induction of the *Slender Rice-1* (*SLR1*) and *SLR Like-1* (*SLRL1*) genes, which lead to the inhibition of GA-mediated shoot elongation. Hence, energy is conserved and used when the water subsides for plant re-growth and survival (Fukao and Bailey-Serres, 2008). Further, efforts were made to identify four non-*Sub1*novel QTL on chromosomes 1, 2, 9, and 12 in moderately tolerant cultivars, IR72 and Madabaru, suggesting that alternative pathways may exist independent of the ethylene-dependent pathway of the *SUB1* gene, and the potential of pyramiding of these QTL with *SUB1* QTL for improved submergence tolerance (Septiningsih et al., 2012).IRRI, Philippines in collaboration with other countries introgressed*SUB1* QTL into eight rice mega varieties using marker-assisted backcrossing strategy and resulted in the development of Swarna-Sub1, Samba Mahsuri-Sub1, IR64-Sub1, BR11-Sub1, Thadokkam 1-Sub1, CR1009-Sub1, PSBRc18-Sub1, and Ciherang-Sub1 (Iftekharuddaula et al., 2011; Neeraja et al., 2007; Septiningsih et al., 2009, 2013, 2015). Many other popular varieties such as Ranjit, Bahadur, Pooja, Gayatri, Varshadhan and Sarala areconvertedas submergence tolerant version (Pradhan et al., 2018). Tolerance for biotic and abiotic stresses *viz*., submergence, and bacterial blight have been improved in the popular rice variety, Swarna (Pradhan et al., 2019a).

4.3 Salinity

Approximately, 100 million ha of global land is affected by salinity in the South and South-east. In India, about 6.73M hectare land is affected with salts encompassing sodicity, inland, and coastal salinity (Sharma et al., 2004b). Therefore, breeding for salt-tolerant rice cultivars with pyramiding of major tolerance QTL/genes is considered to be one of the most economic options to assure food security. More than 638 QTL related to salinity tolerance have been identified in rice (Yang et al., 2020). Initially, a major QTL, *Saltol* associated with seedling stage tolerance and explaining about 40% phenotypic variance was detected on chromosome 1 in salt-tolerant landrace, Pokkali (Bonilla et al., 2002). It controls K^+/Na^+ homeostasis in the shoot. Soda et al. (2013) identified salinity-induced factors (*SIFs)* within the *Saltol* QTL region in the Pokkali, which are putatively associated with vegetative growth, fertility, viability, and early flowering under salinity stress. Later, another major QTL, *SKC1* encoding a sodium transporter (*OsHKT1*) for shoot K^+/Na^+ content was mapped on chromosome 1 in salt-tolerant landrace, Nona Bokra (Ren et al., 2005). *SKC1* (Ren et al., 2005), DST (Huang et al., 2009a), and *qSE3* (He et al., 2019) are three major genes for salinity tolerance in rice. Yang et al. (2020) identified 70 salt tolerance candidate genes through meta-QTL analysis, few QTL reported earlier studies such as *OsHAK5*, *OsGSK1*, *OsDR2*, *OsGRX8*, *RSS1*, *SIT1*, inositol 1,3,4-trisphosphate 5/6-kinase gene calcineurin B-like–interacting protein kinase gene, tandem zinc finger protein, *SALT-RESPONSIVE ERF1*, etc. were validated. FL478 (IR66946-3R-178-1-1) line containing *Saltol* QTL was developed from IR29/Pokkali, and used as an improved donor for introducing salinity tolerance into BR11 and BRRI dhan28 in Bangladesh; AS996 and BT7 in Vietnam; Rassi in West Africa and Pusa Basmati 1121 and PB6 in India through MABC approach. Though *Saltol* and the most of the reported QTL are effective at the seedling stage, hardly any major QTL have been identified for the reproductive stage. Hence, there is a need to explore and utilize other QTL with better potential especially for reproductive stage tolerance (Jaisal et al., 2019).

4.4 Cold

Cold stress severely influences growth and productivity, causing a 5%-10% reduction in yield annually, and occasionally it could go up to 20%-40%. The reproductive stage is more prone to cold stress and significantly reduces the yield potential. More than 490 QTL have been identified for cold stress tolerance during germination, seedling, and reproductive growth stages (Sun et al., 2019a; Gopinath 2020; Yang et al., 2020). Recently, several QTL have been fine mapped, such as *qCTB8, qCTB-7, qAL09-2/qAL10-2, qAL09-3/qAL10-3, qCTR5,qCTR12, qCT-3*-2, and *qLTB3* (Kuroki et al., 2007; Zhou et al., 2010; Tazib et al., 2015; Shimono et al.,

2016; Zhu et al., 2015; Ulziibat et al., 2016). Yang et al. (2020) carried out a met-QTL analysis and identified 70 candidate genes for cold tolerance. Only a few genes, *Ctb1*, *Ctb2,* and *CTB4a* have been cloned (Saito et al., 2010; Zhang et al., 2017a). The mechanism of cold tolerance through different tolerance genes, pathways and transcription factors involved were reported by the comparative RNA-seq analysis in seedling stage chilling stress tolerance in *indica* rice (Pradhan et al., 2019). Eleven QTL, namely *qCTS9*, *qCTS-2*, *qCTS6.1*, *qSCT2*, *qSCT11*, *qSCT1a*, *qCTS-3.1*, *qCTS11.1*, *qCTS12.1*, *qCTS-1b*, and *CTB2* were validated in an association study for seedling stage cold tolerance in *indica* rice and suggested for pyramiding for developing a strongly chilling tolerant variety (Pandit et al., 2017). Fujino et al. (2019) successfully pyramided two QTL, *qSCT1a* and *qSCT2* for cold tolerance.

4.5 High-temperature

High-temperature stress is one of the major threats to rice production (Krishnan et al., 2011; Pradhan et al., 2016b). It causes abnormal pollination, resulting in poor seed set and low grain quality during the reproductive stage (Krishnan et al., 2011). More than 175 QTL have been identified for heat stress tolerance at different stages of rice, such as the seeding and reproductive stages using different mapping populations (Cao et al., 2020; Nubankoh et al., 2020; Chen et al., 2021). These mapping populations have been phenotyped at the time of heading under controlled environment conditions or under high-temperature condition by late planting in the open field. Cao et al. (2020) fine mapped *qHTB1-1* using chromosome-segment introgression line, IL01-15 of *Oryza rufpogon,* and identified two candidate genes (*LOC_Os01g53160,* and *LOC_Os01g53220*) conferring tolerance to heat stress at the booting stage. Chen et al. (2021) fine mapped a major QTL, *qHTT8* in heat-tolerant rice cultivar, Huanghuazhan (HHZ), and identified two candidate genes (*LOC_Os08g07010,* and *LOC_Os08g07440*) conferring tolerance to heat stress at the flowering stage. However, few QTL related to rice HT stress tolerance have been cloned (Cao et al., 2020).

5. IDENTIFICATION AND CLONING OF QTL/GENES FOR YIELD AND RELATED TRAITS

Several agronomic and yield-related traits like plant height, tiller number, grain number, grain weight, grain yield, etc are mainly controlled by QTL. A complete understanding of the function of QTL responsible for different agronomic traits could increase the productivity of crops. Grain yield is a complex trait, controlled by many QTL/genes, and highly affected by the environment (Sharma and Sharma 2007; Ranawake et al., 2013). Improvement of rice grain yield is the main target of breeding programs to develop high-yielding rice varieties (Ranawake et al., 2013). Some of the QTL/genes for different yield-related traits have been effectively used in MAS programs and cloned to understand their biological functions.

5.1 Heading date

Heading date is a complex trait controlled by multiple genetic and epigenetic factors. Many environmental factors such as day length, temperature, light intensity, and nutrients control heading date in rice (Yano et al., 2001). Several QTL/genes responsible for the heading date have been identified. Some well-known proved QTL/genes controlling heading date are *Hd1, Hd2, Hd3, Hd5, Hd6 Hd3, Hd5, Hd6, Hd17, HBF1¸ HAPL1, DTH3, HDR1, OsMADS3, OsMDAS6, OsMADS18, OsMADS22, qDTH-5, qDTH8, Ghd7*, etc. The basic vegetative growth phase and days to flowering are controlled by many identified genes such as *Ef-1* and *Se-1-Se-7* (Poonyarit et al., 1989). *Hd1* present in rice plays a significant role in flowering. Under short-day conditions, *Hd1* promotes floral transition, while suppressing under long-day conditions (Lin et al., 2000). Some of the important cloned genes are *OsMADS3, OsMADS6, HDR1/OsMADS22, DTH3, Hd6, HAPL1/ HBF1, Hd17, Hd1, OsMADS18,* and *Hd5* (Sun et al., 2016; Brambilla et al., 2017; Zhu et al., 2017; Khahani et al., 2020) controlling heading located on chromosomes 1, 2, 3, 6, 7 and 8. Other genes also involved in floral formation. *ETR2* gene is reported to affect flowering time (Wuriyanghan et al., 2009). The *OsbZIP62* is shown to interact with *Hd3a* protein and affect flowers in rice (Brambilla et al., 2017).

5.2 Plant height

Plant height is the main determining factor in plant architecture, which directly affects the harvest index and grain yield potential. It is one of the leading attributes to the 'Green Revolution' introduced by semi-dwarf phenotype and using a large amount of nitrogen fertilizer (Sakamoto and Matsuoka 2008). Several plant height QTL/ genes have been identified using various interspecific, inter-subspecific, and intra-subspecific crosses (You et al., 2006). The dwarf responsive genes, *d-1* to *d-88*, and semi-dwarf genes, *sd-1* to *sd-7* have been identified (Kinoshita 1995; Peng et al., 2014). The cloned genes controlling plant height are *OsSIN, sd1, OsRH2, d10, d11, OsDSS1, OsSIN, d11, OsRH2, OsDSS*1, and *BRD2* (Yano et al., 2015; Huang et al., 2016; Liu et al., 2016; Zhou et al., 2017; Khahani et al., 2020). Semi-dwarf1 (*sd1*) controls gibberellin (GA) synthesis and plant height. It has been extensively utilized in the "Green Revolution" for improving rice yield (Ashikari et al., 2002; Sasaki et al., 2002).

5.3 Tiller number

Tiller number (TN) is one of the most important features in plant architecture and grain production in rice. It is a quantitative trait with a relatively low heritability of 29.8-49.6% (Xiong 1992). Despite its agronomic importance, only a few tillers controlling QTL/genes have been identified and cloned, such as

MONOCULM 1 (*MOC1*), *Dwarf 3 (d3), Dwarf 10 (D10), Dwarf 14 (D14), Dwarf 17 (D17),* high-tillering *Dwarf1 (HTD1), Dwarf27 (d27), OsIAA6, PAY1*, etc (Hussain et al., 2014; Khahani et al., 2020). Tiller number regulator *MONOCULM 1 (MOC1)* is one of the important gene, which increases the formation of axillary buds and tiller numbers (Wang et al., 2018a). *MOC1* is protected from degradation by binding to the *DELLA* protein *SLENDER RICE 1* (*SLR1*). GAs enhances the degradation of *SLR1*, leading to degradation of *MOC1*, and thereby decreasing in tiller number and elongation of the stem (Liao et al., 2019). Lu et al. (2015a) and Tanaka et al. (2015) reported that *MOC3/TILLERS ABSENT 1* (*TAB1*) is also required to initiate the development of an axillary meristem in rice. Oikawa (2009) and Tabuchiet al. (2011) indicated that after axillary meristem formation, the *LAX PANICLE 1* (*LAX1*) and *LAX2* genes are required for the maintenance of axillary development. Khahani et al. (2020) carried out genome-wide meta-QTL analysis for grain yield and related traits including tiller number. A total of 77 QTL for tiller number reported from 101 studies during 1996 to 2018 were placed in 10MQTL regions. Two MQTL were found on chromosomes 1 and 3, while one each on chromosomes 2, 4, 6, 7, 8, and 9. They confirmed the position of few earlier identified and cloned genes using meta-QTL analysis. The genes *d10, OsIAA6,* and *PAY1* controlling tiller number were found in MQTL-TN1, MQTL-TN2, and MQTL-TN9, respectively. A homologous *OsPIN5b* gene at MQTL-TN10 on chromosome 9 was identified, which controls tiller number, plant height, panicle size and other traits related to plant architecture (Lu et al., 2015b). Further, they validated few earlier identified genes, *GSK3/SHAGGY* like kinase, *GA2ox* genes in MQTL-TN1, and *GRAS* TFs in MQTL-TN4 and MQTL-TN7 regions, which regulate tiller number (Hussain et al., 2014).

5.4 Grain and spikelet number

Several QTL for grain and spikelet numbers have been identified (http://www.gramene.org/). Some of them have been fine mapped and cloned. Deshmukh et al. (2010) identified *qGN4.1* for grain number while Singh et al. (2018) introduced it into 12 different high-yielding mega varieties of rice though the MAS program. All the QTL-NILs of 12 mega varieties showed a significant increase in a total number of grains (spikelets) and the number of well-filled grains per panicle. Sasaki et al. (2017) fine mapped *qTSN12.2* present on chromosome 12 and controlled total spikelets per panicle. The QTL, *qSPP2.2* regulating spikelet number per panicle was fine mapped and located on the long arm of chromosome 2 (Kaur et al., 2018). QTL/genes such as *Gn1a, GNP1, DEP1, IPA1/OsSPL14, GAD1, An-1, OsCBL8, OsDim1, OsMADS18, PAY1, SAPK2, DTH7/Ghd7.1, Ghd7, DTH8/Ghd8, GN2, OsSPL16*involved in the regulation of grain number per panicle have been cloned and characterized (Xu et al., 2019; Li et al., 2021; Zhong et al., 2021). Cytokinins and gibberellins play antagonistic roles in modulating the

activity of the reproductive meristem. Up-regulation of gibberellins negatively affects meristem activity, while cytokinin activity leads to increased grain number. *Gn1a* encodes cytokinin oxidase/dehydrogenase (CKX), which catalyzes the irreversible degradation of cytokinins (CKs) (Ashikari et al., 2005). Hence, decreased expression of *Gn1a* in inflorescence meristems can lead to the accumulation of cytokinin, which finally results in increased grain number (Hwang et al., 2012). The mutant allele of *DEP1* encodes a truncated phosphatidyl ethanolamine binding protein-like domain protein and causes erect and dense panicle and high grain number although the panicle length is reduced (Huang et al., 2009a).The *WEALTHY FARMER'S PANICLE (WFP)* QTL, *IPA1/OsSPL14*encodes squamosa promoter binding protein-like 14, which positively regulates panicle branching and grain number at the reproductive stage, while negatively regulate the shoot branching at the vegetative stage in rice (Miura et al., 2010). Further research has demonstrated that *IPA1* is a positive regulator of *DEP1* (Lu et al., 2013). *NOG1* encodes an enoyl-CoA hydratase/isomerase and increases grain yield by enhancing grain number with no negative effects (Huo et al., 2017). Three major QTL *DTH7/Ghd7.1, Ghd7*, and *DTH8/Ghd8* controls the heading date but also increase grain number under long-day conditions (Wei et al., 2010; Weng et al., 2014; Yan et al., 2011; Gao et al., 2014; Liu et al., 2013). *GNP1* encodes gibberellin 20-oxidase, which enhances cytokinin production, thereby enhances grain number (Wu et al., 2016). *GN2* exhibits pleiotropism and influences grain number, plant height and heading date (Chen et al., 2017b). *APO1* along with *Gn1a* increases primary branches, formation of vascular bundles, and grain number (Terao et al., 2010). *Gn1a* and *Dep1* genes were edited through CRISPR/Cas9 technology for enhancing grain yield (Huang et al., 2018). The plants containing *Gn1a* mutant gene showed an increase of grain yield up to 23.8%, whereas *Dep1* mutant gene showed an increase of 51.1% yield (Huang et al., 2018). Two genes, *OsOAT* and *LSSR1* regulate the seed setting rate (Liu et al., 2018; Xiang et al., 2019). *OsSPL16* promotes grain filling and thereby increases the yield in rice (Zhong et al., 2019).*GIF1* is a key gene, which encodes a cell wall invertase (*OsCIN2*) that regulates sucrose transport and unloading, and is required for carbon partitioning during the early grain-filling stage (Wang et al., 2008). *GIF2* encodes an ADP-Glcpyrophosphorylase large subunit, which plays an important role in the regulation of grain filling and starch biosynthesis during caryopsis development (Wei et al., 2017). Liu et al. (2019a) cloned the favorable allele of major grain filling rate QTL/gene (*GFR1*) in rice accession, Ludao.

5.5 Grain weight and size

Grain weight (GW) is one of the important yield components for increasing grain yield. Many QTL/genes related to grain size have been identified. Some QTL/genes are negative regulators, such as *GW2, qSW5/GW5, GS9, TGW6, WTG1,*

OsCKX2, TGW3, GS3, DEP1, qGL3/ OsPPKL1, and *OsSNB* (Liu et al., 2017a; Zhao et al., 2018; Li et al., 2021), and while other QTL/genes *viz.*, *GL2, GL3.1, GS5, GL7, GLW7, GW6a/OsglHAT1, GNS4, GS5/qTGW5a, OsAGSW1, RAG2*, and *GW8 (OsSPL16)* are positive regulators (Wang et al., 2015; Li et al., 2021). Bao et al. (2015) identified three QTL that displayed hierarchical effects on grain length and grain weight in the order of *qTGW3.2 (qGL3), qTGW3.1 (GS3)* and *qTGW3.3*. McCouch et al. (2016) reported several QTL for grain length by GWAS using 210 rice accessions. Park et al. (2021) mapped *OsBRKq1* using Samgang/Nagdong double haploid (SNDH) populations. It controls 1000-grain weight, grain length, grain width, and exhibited similar functions of the brassinosteroid leucine-rich repeat-receptor kinases of *Arabidopsis thaliana* and *Zea mays*. Shi et al. (2020a) identified *OsGASR7/GW6* gene, which found to regulate grain width and weight. Li et al. (2020b) mapped *TGW12* using the RIL population of K1561 of *Oryza minuta* with the cultivar, G1025. The candidate gene, *TGW12* encodes MADS-box protein and increases grain-weight. Zhang et al. (2020a) identified two major QTL, *qGL-5* and *qGL-6*using chromosome segment substitution line Z1392, which increases grain length. They observed a positive epistatic interaction between *qGL-5* and *qGL-6*. Several QTL/genes affecting grain weight and size have been cloned and functionally characterized, such as *GW2* (RING-type E3 ubiquitin ligase), *qTGW3 (GSK3/SHAGGY*-Like Kinase), *qSW5/GW5* (arginine-rich domain nuclear protein), *TGW6* (IAA (indole-3-acetic acid)- glucose hydrolase protein), *GW6a/ OsglHAT1* (GNAT-like protein), *GW8* (*OsSPL16*) (SBP domain transcription factor), *GS3* (transmembrane protein), *GS5/qTGW5a* (serine carboxypeptidase), *OsAGSW1* (ABC1-like kinase), *qGL3/OsPPKL1* (Kelch-like domain), *qHD5* and *OsSNB* (AP2 transcript factor), *WTG1* (WIDE AND THICK GRAIN 1(otubain-like protease), and *OsMAPK6* (mitogen-activated proteinkinase) (Liu et al., 2015b; Li et al., 2021). *GS3* is the first major gene to be cloned, which regulates grain yield. Mutations in *GS3* increase grain length and grain weight, thereby enhancing grain yield (Mao et al., 2010; Zhong et al., 2020; Usman et al., 2021). It has been used to improve yield with the CRISPR-Cas9 system (Zeng et al. (2020). Mutations in the first exon of *GS9* using CRISPR/Cas9 technology disrupted the normal expression of *GS9*, resulting in increased grain length (Zhao et al., 2018). QTL for submergence tolerance and yield component were transferred to the popular variety, Swarna and Maudamani through marker-assisted breeding (Mohapatra et al., 2021; Pandit et al., 2021).

5.6 Panicle architecture/ traits

Panicle traits are among the most important agronomic characters, which are directly related to grain yield in rice. Many QTL/genes associated with panicle architecture have been identified, cloned, and characterized at the molecular level. Panicle length (PL) is inherited in a quantitative manner and controlled by both

major and minor QTL (Liu et al., 2011). These QTL were commonly co-identified with heading date. In general, subspecies *indica* has a longer panicle length and looser spikelet density than those of *japonica*. To date, at least 270 QTL for panicle length have been detected. Zhang et al. (2015) identified two QTL, *qPL6* and *qPL8* in the BIL population derived from the cross of Nipponbare (*japonica*) and WS3 (*indica*). Further, *qPL6* and *qPL8* were found to show additive effects for panicle length. Yoshida et al. (2013) reported that *TAWAWA1* (*TAW1*) encodes an ALOG transcription factor, which regulates a number of secondary branches. *IPA1/WFP (OsSPL14*) promotes primary branching but represses tiller formation (Lu et al., 2013). A major locus, *LONG PANICLE 1 (LP1)* on chromosome 9 was identified via both linkage and association mapping. Sequencing analysis of *LP1* in two parents and 103 rice accessions indicated that SNP1 is associated with panicle length. The *LP1* allele of Xiushui79 leads to reduced panicle length, whereas the allele of C-bao increases panicle length. Sun et al. (2017) identified *PL6-5*, *PBN6-1*, and *PBN6-2*. The major QTL, *PL6-5* for panicle length was detected to be consistent in all the four populations and in two locations.*OsSPL7* and *OsSPL17* show similar effects on panicle development. *GRAIN SIZE AND NUMBER1* (*GSN1*) affects the number of secondary branches and grain size (Guo et al., 2018b). Jiang et al. (2018) found that transcription factor, *NAC* (*OsNAC2*) controls primary branches *via* an *IPA1-DEP1*-related pathway. Kim et al. (2018) pyramided *Gn1a-type 3* and *OsSPL14 WFP* alleles, and observed increased grain number and thereby grain yield. *GNP6*, a novel allele of *MOC1* regulates panicle and tiller development. The mutant genes, *moc1-1, moc1-3, moc1-5*, and *moc1-6/gnp6* show defects in panicle branch formation (Zhang, 2021). Wang et al. (2020a) pyramided *GNP1* and *NAL1*, which showed increased grain number. However, undesirable effects are produced by some genes, i.e., *Ghd7* delays flowering time; *IPA1* reduces tiller number and panicle branching, while *FZP* reduces grain size (Wang 2015). Stacking of QTL for submergence tolerance and yield component (*OsSPL14*) in the popular variety, Swarna showed increased yield (Mohapatra et al., 2021). The lax panicle mutants such as *lax1*, *lax2* (*gnp4*), and *moc1* (*gnp6*) produce large grains. Many QTL/ genes involved in panicle branching such as *Gn1a, IPA1/ OsSPL14, OsSPL17, LAX1, DEP1, EP2/DEP2, DEP3, EP3/LP, FZP, MOC1, TAW1, OsOTUB1, IPA1, IPI1*, and *OsNAC2*have been identified and cloned(Li et al., 2021). The identification of elite alleles of core genes and utilization in molecular breeding programs would improve grain yield.

5.7 Grain yield *per se*

Grain yield in rice has always remained as the main breeding objective. Zhaobin et al. (2010) mapped two QTL from wild rice, *qGYP-2-1* and *qGYP-3-1* on chromosomes 2 and 3 which increased the yield of Yuexiangzhan by 40.05% and 49.04%, respectively. Swamy et al. (2014) carried out the advanced backcross

QTL (AB-QTL) analysis in two *Oryza nivara* derived BC_2F_2 populations and identified 28 QTL in population 1, and 26 QTL in population 2 for all the nine traits evaluated. The two most significant yield-enhancing QTL, *yldp9.1* and *yldp2.1* showed an additive effect of 16 and 7 g per plant in population 1, while, *yld 11.1* showed an additive effect of 10 g per plant in population 2. At least one *O. nivara*-derived QTL with a phenotypic variance of >15 % was detected among the 7 traits.The *O. nivara*-derived QTL *ph1.1, ntl2.1, nsp1.1, nfg1.1, bm11.1, yld2.1* and *yldll.l* were found to be conserved at the same chromosomal locations in both the populations. Two major QTL clusters were detected at the marker intervals RM488-RM431 and RM6-RM535 on chromosomes 1 and 2, respectively. Khahani et al. (2020) carried out a MQTL analysis using 165 QTL reported from 101 studies conducted during 1996 to 2018 using 122 bi-parental populations phenotyped under unstressed conditions.They identified a total of 19 MQTL regions. The MQTL regions 5, 2, 4, 2, 1, 1, 2, 1, and 1, were located on chromosomes 1, 2, 3, 4, 6, 7, 8, and 11, respectively. The number of QTL identified were found to be 16, 7, 10, 4, 3, 3, 5, 2, and 3, on chromosomes 1, 2, 3, 4, 6, 7, 8, and 11, respectively. The earlier identified genes such as *GIF2, OsLSK1, d11, APO1, DEP3,* and *OsSPL13* controlling grain yield were located on MQTL-YLD3, YLD4, YLD13 YLD14, and YLD15 regions, respectively. They also identified few candidate genes such as *Os01g0171000* gene in MQTL-YLD1 which encodes BRASSINOSTEROID INSENSITIVE 1-associated receptor kinase and *SWEET15*genein MQTL-YLD6for MQTL regions or grain yield in rice (Yang et al., 2018).

5.8 Lodging resistance

Lodging is one of the major problems for reducing grain yield and quality (Shah et al., 2019). Culm diameter and size are highly correlated with the lodging resistance. Lodging resistance is a complex quantitative trait, which is affected by many factors, such as the culm morphology, the culm quality, and the external environmental conditions. Many QTL associated with traits relating to lodging resistance have identified, such as *prl5* (Kashiwagi et al., 2006), *SCM1* and *SCM2* (Ookawa et al., 2010*), qPRF-8* (Hu et al., 2008), *lrt5* (Ishimaru et al., 2008), *SCM3* and *SCM4* (Yano et al., 2015) and BSUC11 (Kashiwagi et al., 2016). Few QTL controlling lodging resistance and grain yield simultaneously have also been identified. Strong culm2 (*SCM2*) was identified in *indica* variety, Habataki, and introgressed into Japanese elite variety, Koshihikari which enhanced culm strength and grain yield (Ookawa et al., 2010). ABERRANT PANICLE ORGANIZATION1 (*APO1*) is similar to *SCM2* for enhancing culm strength but it also controls panicle morphology, and increases spikelet number (Ikeda-Kawakatsu et al., 2009). Yano et al. (2015) isolated *SCM3*, which is identical to rice TEOSINTE BRANCHED1 (*OsTB1*) and FINE CULM 1 (*FC1*). *OsTB1* positively controls strigolactone (SL) (Minakuchi et al., 2010). Two pleiotropic QTL genes, *SCM2* and *SCM3* increase

the culm strength and spikelet number without causing panicle deformities. Yadav et al. (2017) identified 12 QTL for lodging resistance in direct-seeded rice and identified gibberellic acid 3 b-hydroxylase as a candidate gene. The locus, *prl5* delays leaf senescence and increases the carbohydrates in the lower part of the stem increases lodging resistance (Kashiwagi and Ishimaru 2008). Kashiwagi et al. (2008) identified four QTL, *sdm1, sdm7, sdm8*, and *sdm12* associated with increasing the stem diameter and lodging resistance Jian et al. (2019) identified 19 QTL on chromosomes 2, 4, 6, 8, 9, and 10 using RIL mapping population. Long et al. (2020) identified 12 QTL for stem diameter, 11 QTL for stem length and 3 QTL for breaking strength using backcross derived lines of 93-11 and *O. longistaminata*. A total of 12 QTL were identified in *O. longistaminata* among which *qLR1,* a major QTL increased stem diameter, stem length, and breaking strength. Also, another major QTL, *qLR8* significantly enhanced the breaking strength. Guo et al. (2020) performed GWAS using a panel of 524 accessions and identified 127 QTL associated with lodging resistance. A positive correlation between culm strength and panicle weight, while a negative correlation between culm strength and panicle number was observed. Zhao et al. (2021) identified QTL for pushing strength of the lower stem before the heading date (PSLSB) and pushing strength of the lower stem after the heading date (PSLSA) on chromosome 6 between RM439 and RM20318. They identified 15 lodging resistance candidate genes. The candidate gene, *Os06g0623200*, named *OsPSLSq6*, is similar to Cinnamoyl-CoA reductase. It involves in lignin biosynthesis in defense responses. Genes encoding extensins (Fan et al., 2018b) and sucrose synthases (Fan et al., 2017) have impacted on cell wall construction and thus culm physical strength. Genes responsible for silicon accumulation increases resistance to lodging. The silicon transporter genes, namely, *Lsi1*, *Lsi2,* and *Lsi6*have been identified, (Ma et al., 2006; Ma et al., 2007; Yamaji and Ma 2009).

6. GENE PROSPECTING AND ALLELE MINING

Development of superior and high-yielding varieties made possible by the accumulation of beneficial/ superior alleles from vast plant genetic resources existing worldwide. Still, a significant portion of these beneficial/ superior alleles was unused and left during evolution and domestication. Introducing novel alleles from wild crop plants to the cultivated varieties has clearly demonstrated that certain alleles and their combinations potentially make dramatic changes in trait expression. Hence, the vast germplasm resources need to be relooked for novel alleles to further enhance the genetic potential of crop varieties for various agronomic traits. Gene prospecting and allele mining aim at identifying allelic variation of relevant traits within genetic resource collections. Allele mining helps in tracing the evolution of alleles, identification of new haplotypes and development of allele-specific markers for use in marker-assisted selection. It is

important to use this genomic information for the identification and isolation of novel and superior alleles of agronomically important genes from crop gene pools to suitably organize for the development of improved cultivars. The 3K genomes dataset provides an efficient means to map traits and validate certain target sequences. Known sequences can be used to search the genome database, and identify accessions with unique sequences and haplotypes. In allele mining, different software are used for identifying the nucleotide variation and prediction of amino acid changes which is responsible for encoding protein structure and functions. Some of the bioinformatics software are ClustalW, DCPD, Fast PCR, and Plant CARE. Allele mining can be effectively used for the discovery of superior alleles are through 'mining' the gene of interest from diverse genetic resources. According to Imam et al. (2016), a list of 338 rice landraces phenotyped in the rice blast nursery at Central Rainfed Upland Rice Research Station, Hazaribag. Six of them were further selected based on their resistance and susceptibility pattern for virulence analysis and selective pattern study of the *Pi9* gene. Zhang et al. (2016a) performed allele mining for rolled leaf trait considered to be a key component of plant architecture, with representative SNPs, and identified the elite germplasms for the improvement of rolled leaf trait. Most favorable alleles at the detected loci were contributed by various 3K Rice germplasms. By a re-scanning of the candidate region with more saturated SNP markers, they dissected the region harboring *gRl4-2* into three sub-regions, in which the average effect on LRI was 3.5% with a range from 2.4 to 4.1% in the third sub-region, suggesting the presence of a new locus or loci within this region. Rice yellow mottle virus (RYMV) is a major rice pathogen in Africa. Three resistance genes, i.e. *RYMV1*, *RYMV2* and *RYMV3* encodes the translation initiation factor *eIF (iso) 4G1,* and the best candidate genes for *RYMV2* and *RYMV3* encode a homolog of an *Arabidopsis* nucleoporin (CPR5) and a nucleotide-binding domain and leucine-rich repeat-containing domain (NLR) protein, respectively. High resistance is very uncommon in Asian cultivated rice (*Oryza sativa*), with only two highly resistant accessions identified so far, but it is more frequent in African cultivated rice (*Oryza glaberrima)*. A resistance survey was done in a reference collection of 268 *O. glaberrima* accessions. A total of 40 resistant accessions were found, thus confirming the high frequency of resistance to RYMV in this species. The variability of resistance genes or candidate genes in this collection was based on either high-depth Illumina data or the Sanger sequencing. Alleles previously shown to be associated with resistance were observed in 31 resistant accessions but not in any susceptible ones. Five original alleles with a frameshift or untimely stop codon in the candidate gene for RYMV2 were also identified in resistant accessions. Genetic analysis revealed that these alleles, as well as T-DNA insertions in the candidate gene, were responsible for RYMV resistance. All the 40 resistant accessions were ultimately linked to a validated or candidate resistance allele at one of the three

resistance genes to RYMV (Pidon et al., 2020). Mishra et al. (2016) performed haplotype analysis and revealed the haplotypes of H5 and H1 for HKT1; 5 and HKT2; 3, respectively associated with high salinity tolerance. This is the first study of allele mining of eight members of the HKT gene family from Indian wild rice reporting a salt-tolerant allele of *HKT2*; 3. *HKT1*; 5 also showed a salt-tolerant allele from wild rice. Phylogenetic analysis based on the nucleotide sequences showed the difference in grouping of the HKT family genes compared to the prevailing protein sequence-based classification. Singh et al. (2014) identified three coding SNPs and one haplotype identified in the *OsDREB1F* gene and has the potential to be associated with drought tolerance in rice. Samal et al. (2014) studied a cluster of three ethylene response factor (ERF) like genes at the *Sub1* locus on chromosome 9 are known to confer tolerance to complete submergence for about two weeks. With an objective to identify superior alleles, haplotyping of *Sub1* locus in fifty selected rice accessions using a set of gene-specific markers for *Sub1A* and *Sub1C* genes was conducted. In these accessions, ten haplotype patterns having different combinations of genes were identified. Hittalmani et al. (2013) studied on the marker RG64 linked to *Pi-2*, a major dominant blast resistance gene on chromosome 6, and marker pTA248 on chromosome 11 linked to *Xa21*, a resistance gene to bacterial leaf blight were used to detect the presence of resistance alleles. Three different types of bands of 1 kb carrying *Xa21* resistance allele and two susceptible alleles of 700 and 750 bp were amplified using pTA248. A total of 28 genotypes carried resistance alleles for both blast and bacterial leaf from among them Gowri Sanna, Ponni, Antharsali, and Doddabyranellu were popularly preferred by the farmers. These can serve as donor lines for transferring both resistance genes simultaneously.

7. ROLE OF TRANSCRIPTOMICS IN BIOTIC AND ABIOTIC STRESSES

The transcriptome of rice, which represents the set of transcripts, is very dynamic and guided by the time, tissue or cell, and growth conditions. Rice, which is grown under different ecologies, is subjected to multiple stresses and conditions, the dynamics of its transcriptome changes both over the time and space scale. Transcriptome analysis has been widely studied in rice using different tools, the most prominent among them are the hybridization microarray, and sequencing-based HT/RL SAGE and RNA Seq using NGS technologies, and the findings from these studies have been used successfully to improve rice (Song et al., 2018). Understanding of rice transcriptome helps in deciphering the mechanisms of different biological processes under the given/ different environments, and also to identify/ confirm the candidate genes having a role in various functions, such as tolerance/resistance to biotic and abiotic stresses, growth, and development, morphological and yield-related traits, nutritional quality traits, etc. (Sharma et al., 2016; Pradhan et al., 2019; Ren et al., 2020).

7.1 Biotic stresses

Rice is exposed to many biotic stresses imposed by pests and diseases. Therefore, comparative transcriptome analysis is most commonly used to decipher the mechanism of the stress response (Sharma et al., 2016). Transcriptome analysis was extensively used to understand the rice-*Magnaporthe oryza*e interaction. Among the earliest reports, Matsumura et al. (2003) used the modified SAGE (Super SAGE) technique, and the gene expression profiles from both rice and the blast fungus identified the hydrophobin gene as the highly transcribed fungal gene in all the blast infected leaves of rice. In the later studies using the microarray, the transcriptome analysis of two transgenic rice lines overexpressing *OsWRKY13* (*D11UM1-1* and *D11UM7-2*) and respective control plants (Mudanjiang 8), Qiu et al. (2007) identified a set of 460 up-regulated and 478 down-regulated genes specific to *D11UM1-1*, while 445 up-regulated and 605 down-regulated genes specific *to D11UM7-2*. The RNAseq analysis in rice has revealed the transcriptional activation of genes coding for kinases, MAPK, production, and detoxification of ROS, LRR signaling proteins, transcription factors belonging to *MYB,WRKY*, *NAC,DOF*, and *ERF* family, Jasmonic acid and ethylene hormones, enzymes such as glycosyl hydrolases and chitinases, and those governing pathogenesis, lipid biosynthesis, and secondary metabolism (Zhang et al., 2016b; Jain et al., 2017; Kumar et al., 2021).

Transcriptome analysis was used to identify the putative regulators of sheath blight response in rice and studies showed a different set of DEGs in resistant and susceptible rice lines challenged with *R. solani* (Zhang et al., 2017b; Shi et al., 2020b). Further, it was found that the metabolic pathways are common between the resistant and susceptible lines, but it was a time scale difference in the resistance response (Zhang et al., 2017b). Also, it was observed that the expression of genes for TFs, such as *MYB*, *WRKY*, and *NAC*, and transporter, like *MFS*, *ABC*, and *SWEET* were significantly changed due to *R. solani*AG1-IA inoculation (Yuan et al., 2020). Yuan et al. (2020) also found that *OsWRKY53* and *OsAKT1* act as negative regulators of the defense response. Bacterial blight resistance was analyzed at the transcriptome level in order to decipher the underlying molecular mechanism. The rice line CBB23 containing *Xa23* was inoculated separately with virulent and avirulent *Xoo* strains, and found that a total of 1,235 genes were DEGs, and which belonged to biological process, molecular function, and cellular component (Wang et al., 2019a). A similar study identified a set of differentially expressed genes coding for resistance genes, signaling, proteolysis, and phenylpropanoid biosynthesis (Cheng et al., 2016).

Ji et al. (2016b) identified up-regulation of genes coding for WRKYs, MAP3Ks, and WAK in the bakanae disease resistant rice genotype 93-11, and

those coding for phosphorylation and TFs were enriched in rice line Zerawchanicakaratals (Cheng et al., 2020). A recent RNAseq study in rice subjected to tungro disease found that a set of 959 transcripts, coding for cellular pathways governing the stress response, and hormonal homeostasis were differentially expressed in rice lines (Kumar and Dasgupta 2020).

Besides diseases, insect pests are also the major constraints to rice production. Nematodes like *Meloidogyne incognita* and *Meloidogyne graminicola* infect rice roots and cause significant yield loss. Zhou et al. (2020) used RNAseq analysis and found 952 and 647 DEGs after 6- and 18-days post-inoculation (dpi), respectively. Similarly, the transcriptome analysis of rice infected with nematode *Hirschmanniella mucronata* identified 252 DEGs in susceptible lines compared to the resistant line (Sun et al., 2019b). The comparative transcriptome analysis in rice lines infested with BPH and SSB identified DEGs coding for various signaling pathways, phytohormones, rice-pathogen interaction, secondary metabolites, ROS, and TFs (Li et al., 2020c).

7.2 Abiotic stresses

Climate change-driven abiotic stress is the major concern for rice researchers as it is evolving as a major threat to the global food grains production, including rice. Transcriptome analysis is the key tool to decode the molecular mechanism governing the tolerance or susceptible response. Among the abiotic stresses, drought, salinity, high-temperature, cold, flood, and heavy metals are the major constraints to rice cultivation. The comparative transcriptome study involving drought susceptible and tolerant rice cultivar Zhenshan97 and cultivar IRAT109, respectively identified 436 DEGs. The KEGG analysis revealed a positive relationship between the DEGs and photosynthesis function, which act as the significant factor for rice adaptation to drought (Zhang et al., 2016). The transcriptome analysis of root transcriptome of rice NILs in the genetic background of IR64 and with contrasting drought response revealed that the number of DEGs increased with increasing level of drought stress (Moumeni et al., 2011). They also found that DEGs coding mostly associated with secondary metabolism, response to stimulus, amino acid metabolism, transcription and signal transduction, defense response were up-regulated, while DEGs of photosynthesis and cell wall growth were down-regulated (Moumeni et al., 2011).

Salinity is another critical factor affecting rice production. The transcriptome analysis of salt-tolerant and salt-sensitive rice genotypes Xian156 and IR28, respectively identified 1,375 novel genes, and 1,371 of them were annotated genes. They also identified 5,273 DEGs, and 286 of these DEGs were specific to Xian156 (Wang et al., 2018b). Using the microarray database, a seedling stage meta-analysis was performed using different rice genotypes, and 5559 DEGs were identified, and 3210 of these DEGs were specific to the recovery phase (Kong

et al., 2019). Similarly, the comparative transcriptomes of drought-tolerant and salinity-tolerant rice lines, Nagina 22 and Pokkali, respectively with that of IR64 (susceptible to both stresses) identified 801 and 507 DEGs specific to N22 and Pokkali, respectively. In that, TFs from NAC and DBP were specific to N22, while those from bHLH and C2H2 were specific to Pokkali (Shankar et al., 2016). Recently, a transcriptome analysis of rice lines Dongdao-4 and Jigeng-88 with differences in the saline-alkaline stress tolerance identified 3523 and 4066 DEGs in Dongdao-4 and Jigeng-88, respectively. Further, genes with GO terms like response to stress, response to JA, organic acid metabolic process, iron homeostasis, and nicotianamine biosynthetic process were found enriched (Li et al., 2020b). RNA seq analysis is also being used to study the single gene-induced response in rice for salinity tolerance. Very recently, Wang et al. (2021) performed transcriptome analysis of transgenic rice expressing *OsDRAP1* gene and its wild type plant and reported the transgene imparted induction of superoxide dismutase (SOD), malondialdehyde (MDA), and catalase (CAT) coding genes for enhanced redox homeostasis and associated membrane stability during salt stress.

The rising global temperature is posing a great challenge to rice cultivation, which is generally associated with the rice growing season (Dwivedi et al., 2015). Transcriptome studies were useful in understanding the tolerance response and identification putative genes governing heat tolerance in rice. One of the studies consisting of heat-tolerant- Huanghuazhan and heat-susceptible- IR36 grown at 40°C or 32°C identified a total of 4,070 heat stress-responsive (HTR) genes, and 1,688 and 707 of these genes were specific to heat-resistant-cultivar and susceptible-cultivar, respectively. They also found that enhanced signal transduction of endogenous hormones might be the reason for heat tolerance (Wang et al., 2019b). In another study consisting of meta-data, the rice meta-transcriptome analysis revealed a set of 6,956 abiotic stress-tolerant (ASTR) genes, key hub-regulatory genes, and TFs (Smita et al., 2020). The transcriptome analysis of rice subjected to different abiotic stresses leads to the identification of a large number of genes responsible for stress tolerance (Borah et al., 2017). Gene ontology for seedling stage cold tolerance showed the role of transcription regulation, oxygen, lipid binding, catalytic and hydrolase activity for tolerance response while the absence of photosynthesis related genes, starch, fatty acids, and terpenes during the stress were detected in sensitive genotype (Pradhan et al., 2019).

8. ROLE OF MICROMICS (MIRNAS) IN BIOTIC AND ABIOTIC STRESSES, AND YIELD-RELATED TRAITS

MicroRNA is a small single-stranded non-coding RNA (20-24 nucleotides) found in plants, animals, and some viruses, which functions as RNA silencing and post-transcriptional gene expression regulation. MiRNAs function via base-pairing with complementary sequences within mRNA molecules either through cleavage of

mRNA strand or its translation inhibition (Bartel, 2009; Fabian et al., 2010). The biogenesis of plant miRNAs involves transcription of miRNA genes (MIRs genes), processing of primary miRNA transcripts by DICER LIKE proteins into mature miRNAs, and loading of mature miRNAs into ARGONAUTE proteins to form miRNA-induced silencing complex (miRISC). The expressions of MIR genes are crucially regulated by *Cis*-regulatory elements and *trans*-acting regulators (Song et al., 2019). These are transcribed by the DNA-dependent RNA Polymerase II enzyme (Borges and Martienssen, 2015). Plants have evolved miRNAs to modules biotic and abiotic stress-tolerance responses, and some of which are evolutionarily associated with plant environmental adaptation (Song et al., 2019). To date, several methods have been developed to identify or detect the miRNA in plants. Real-time reverse transcription PCR for specific miRNA (qRT-PCR), microarray, and the newly introduced next-generation sequencing (NGS) method are used to detect or identify the miRNAs in plants. NGS is not only suitable for profiling of known miRNAs (as qRT-PCR and microarray can do too), but it also can detect unknown miRNAs for which the other two methods qRT-PCR and microarray are incapable to do. Further, qRT-PCR and Northern blotting were used for further validation of identified miRNAs through the next-generation sequencing (Illumina sequencing) method.

In recent years, miRNAs have been reported to control varieties of biological processes, such as plant development, differentiation, signal transduction, or stress responses, and yield. In response to abiotic stresses such as drought, salt, cold, heat, low light, and nutrient limitations, the expression levels of some miRNAs change, resulting in a modulation of the expression patterns of miRNA target genes that are associated with stress adaptations. A better understanding of the regulation of miRNAs and targets during stress responses can contribute to rice breeding for improving yield, quality, and tolerance to biotic and abiotic stresses.

8.1 Biotic stresses

Biotic stress has become a major concern due to the continuous and fast evolution of biotic agents such as viruses, bacteria, fungus, insects, and nematodes. AGO1 is targeted by *miR168* to confer resistance against *Botryosphaeria dothideain, Malushupehensis* (Yu et al., 2017). The *AGO18* sequester *miR168* in viral response and confers resistance in rice (Wu et al., 2015b). Overexpression of *miR7695* has been shown to confer resistance to infection by the rice blast fungus *M.oryzae* (Campo et al., 2013). Over-expression studies of *miR398b* in rice showed resistance against blast fungus in pre-and post-invasive defense response, whereas, *miR169* accumulation suppresses the expression of target NF-YA (defense responsive transcription factors) genes (Li et al., 2017).

8.2 Abiotic stresses

8.2.1 Cold responsive miRNAs

In sugarcane and rice, cold stress induces the evolutionarily conserved *miR319*. Overexpression of *miR319* down-regulates *TEOSINTE BRANCHED1, CYCLOIDEA, PROLIFERATING CELL NUCLEAR ANTIGEN BINDING FACTOR* genes and enhances cold tolerance in both species (Thiebaut et al., 2012; Yang et al., 2013), suggesting that *miR319* acts as a positive regulator of cold tolerance. Cold-inducible *miR393* also positively regulates cold tolerance in switchgrass by targeting the auxin receptor gene *TIR1/AFB*. Overexpression of *miR393* or mutation of *TIR1/AFB* enhances cold tolerance, which is accompanied by increased expression of cold-responsive genes (Liu et al., 2017b). *miR-319* was down-regulated under cold stress and ABA plays a predominant role in the cold response process. Further *miR-444a* was found to be down-regulated during cold stress (Lv et al., 2010).

8.2.2 Drought responsive miRNAs

Plants modify their root architecture through inhibition of primary root growth and by increasing the development of lateral roots to adapt drought stress (Gilbert and Medina, 2016). In rice, several miRNA-mediated genes were expressed to adapt under drought stress (Singh et al., 2020). *miR165/166* act as important regulators of plant growth and development via targeting transcripts encoding HD-ZIPIII and down-regulation of *miR165/166* shows enhanced drought tolerance in rice and *Arabidopsis* (Yan et al., 2016; Zhang et al., 2018). The expression of *miR393*, *miR319* and *miR397* in response to drought stress was first reported in *Arabidopsis* (Sunkar and Zhu 2004). *miR398* regulates the expression of Cu/ZnSODs that modulates the SOD activity under drought stress in rice (Balyan et al., 2017). miRNAs, *miR531, miR827, miR8175, miR977, miR6300, miR1861, miR440, miR9773, miR3982,* and *miR1876*were also regulated by drought stress in traditional rice landraces (Singh et al., 2020). Zhou et al. (2010) also reported 30 miRNAs, of which 11 were down-regulated and 8 up-regulated under drought stress. Mutum et al. (2016) also reported 71 novel miRNAs in a drought-tolerant variety, Nagina 22.

8.2.3 Salinity responsive miRNAs

Several salt-responsive miRNAs have been discovered and validated in rice such as overexpression of *osa-MIR396c* (Gao et al., 2010) and *osa-MIR393* (Gao et al., 2011) decreases their salt tolerance, signifying the negative roles of these miRNAs for salt tolerance in rice. Transgenic rice overexpressing *miR393* showed an increase in tillers and early flowering, together with decreased tolerance to salt and hypersensitiveness to auxin (Xia et al., 2012). Further, *miR169 g*, *miR169n,* and *miR169o*selectively cleave and transiently inhibit a CCAAT-box-binding TF

gene *NF-YA-8* or *OsHAP2E* under salt stress in rice (Zhao, et al., 2009). Macovei and Tuteja (2012) identified osa-*MIR408, osa-MIR164e,* and *osa-MIR414*, which regulate salt tolerance negatively. Up-regulation of *osa-miR1425* and *osa-miR827b* in rice panicles during salinity indicates that they might be involved in the salinity tolerance of rice (Jeong et al., 2011). Conserved and novel miRNAs that expressed differentially in root and/or shoot tissues targeted transcription factors like AP2/EREBP domain protein, ARF, NAC, MYB, NF-YA, HD-Zip III, TCP and SBP are reported to be involved in the salt tolerance. One of the novel miRNAs, *osa-miR12477*, targeted L-ascorbate oxidase (LAO) shows a build-up of oxidative stress in the plant upon salt treatment, which was confirmed by DAB staining (Parmar et al., 2020).

8.2.4 Yield related traits miRNAs

In rice, the important yield component traits are tiller number, panicle branching, and grain size. Plant miRNA, named *miR156*, was (highly expressed) found to control the expression of Squamosa Promoter Binding Protein-Like (*IPA1/OsOSPL14*) genes involved in the regulation of plant architecture (Xie et al., 2006; Wang, 2016). *OsmiR156* controls the spatio-temporal expression of tillering-related genes such as *TB1*, *LAX1,* and *DWARF 53*, a negative regulator of *TB1* (Liu et al., 2019b). Up-regulation of *OsmiR393* resulted in targeted repression of two auxin receptor genes, *OsTIR1,* and *OsAFB2*, which increased productive tillers and early flowering, hence enhancing yield (Xia et al., 2012). Abnormal inflorescence without the secondary branches was seen in short-height transgenic rice plants by over-expressing *miR396* (Gao et al., 2015). Over-expression of *OsmiR396a* produced dwarf plants with smaller leaves and defective inflorescence architecture that included abnormal panicles, and spikelets with a large proportion of rare conjoined-twin florets, resulting in decreased rice yield (Diao et al., 2018). *miR529* was shown to express preferentially at the reproductive stage, especially during panicle formation and post-embryonic stage (Jeong et al., 2011). *OsmiR535* targeted the expression of *OsSPL7/12/16* and other downstream panicle-related genes, such as *OsPIN1B*, *OsDEP1*, *OsLOG,* and *OsSLR1* (Sun et al., 2019c). In contrast to all other reports on miRNAs, which involved increasing rice yield by repressing the target miRNA, Zhang et al. (2017c) showed that over-expression of *OsmiR408* positively regulated grain yield in rice by increasing panicle branches, and a number of effective grains per the main panicle by down-regulating its downstream target, *OsUCL8*, which is an uclacyanin (UCL) gene of the phytocyanin family. Grain size and panicle branching were improved in transgenic rice plants carrying a disruption of the *miR396*-targeting site in *OsGRF8* as well as by knockout of *miR396e* and *miR396f* (Zhang et al., 2020b). In another study by Zhao et al. (2019b), down-regulation of *OsmiR1432* released its target gene rice Acyl-CoA thioesterase (*OsACOT*) from suppression and promoted grain-

filling in rice, resulting in heavier grains and improved grain yield by at least 17%. Repression of *OsmiR159* caused an elevated abundance of its two targets genes, *OsGAMYB* and *OsGAMYBL1* (*GAMYBLIKE 1*), and negatively impacted multiple agronomic traits including grain size by suppressing cell division, suggesting that *miR159* levels can be modulated to achieve increased grain size and high yield in rice (Zhao et al., 2017).

8.2.5 Light responsive miRNAs

miR156, the most evolutionarily conserved miRNA, targets a subset of *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*IPA1/OsOSPL14*) genes (Axtell and Bowman, 2008). *miR156-SPLs* acts as a negative regulator of the shade-avoidance syndrome, an adaptive strategy of plants to avoid shade from the canopy or compete for light with their neighbors (Casal, 2012). In *Arabidopsis*, shade can activate PHYTOCHROME INTERACTING FACTORs that directly bind to and transcriptionally repress the expression of *MIR156s,* leading to up-regulation of *IPA1/OsOSPL14*. *IPA1/OsOSPL14* mediates diverse morphological changes that are associated with enhanced shade-avoidance syndrome responses (Xie et al., 2017). In rice, *miR2118* targets a long noncoding RNA, PHOTOPERIOD-SENSITIVE GENIC MALE STERILITY 1 TRANSCRIPT (*PMS1T*) and triggers the production of phasiRNAs under long-day conditions, leading to photoperiod-sensitive male sterility (plant mutant whose male fertility is regulated by photoperiod, generally as male-sterile under long-day conditions but fertile under short-day conditions). A single-nucleotide polymorphism from the locus encoding PMS1T might mediate the recognition of PMS1T by *miR2118* and photoperiod-sensitive male sterility in Nongken 58S, valuable germplasm that is initially used in the two-line hybrid rice breeding (Fan et al., 2016).

9. ROLE OF PROTEOMICS IN BIOTIC AND ABIOTIC STRESSES, AND YIELD-RELATED TRAITS

Proteomics studies have been progressed enormously during the last two decades which enabled in establishing and understanding the development of proteomes of various tissues, organs, and organelles under various growth and developmental conditions, and different abiotic and biotic stresses in rice. Proteomics studies in rice have been performed mostly using gel based (2DE in conjunction with IPG, and DIGE) and gel free approaches, especially LC-MS/MS or *iTRAQ* (isobaric tags for relative and absolute quantitation) labeling and detection/identification of protein through MALDI-Toff mass spectroscopy and bioinformatics resources in plant proteomics have move forward the advance in rice proteomics analyses.

Proteomics of major abiotic stresses such as drought, salinity, high and low-temperature stresses, heavy metal stresses, and plant biotic stresses with a special focus on fungal pathogens and *Fusariu*m head blight disease, plant root

proteome response to abiotic stresses, plant phosphoproteomics, redox proteomics, S-nitrosoproteomic, subcellular proteomics under stresses, chloroplast proteome under abiotic stresses, crop proteomics, stress responses of major crops, including rice maize, wheat, barley, soybean, common bean, Solanaceae species, etc have been studied extensively (Kosová et al., 2018).

9.1 Biotic stresses

Xanthomonas oryzaepv. oryzae (Xoo) causes bacterial blight, which is the most important disease of rice caused by bacterial pathogens.Thaumatin-like protein (PR5), probenazole (PBZ), Domain of Unknown Function 26 (DUF26), and â-1,3-glucanase were reported as the key findings in the early studies. A secretome analysis against *Xoo* identified virulence-associated factors and plant-specific proteins such as proteases or peptidases and proteins involved in host defense, the transport system, and maintaining redox balance. *Pseudomonas* is a PGPR used as a biocontrol agent against rice disease, due to its antagonism towards other bacteria and fungi. Analysis of roots and root exudates of rice infected with *Pseudomonas putida* by high-performance liquid chromatography (HPLC) revealed the induction of SA. In another study, *Pseudomonas aeruginosa* was found to produce compounds associated with systemic acquired resistance (SAR), including siderophores (1-hydroxy-phenazine, pyocyanin, and pyochellin), and antibacterial compounds, including 4-hydroxy-2-alkylquinolines and rhamnolipids (Vo et al., 2021). *Magnaporthe oryzae*, the causal agent of rice blast disease, cause huge losses in rice. The proteomics studies have revealed the roles of DUF26, nucleotide binding-leucine rich repeat, PRs, ROS production-scavenging enzymes, heat shock proteins (HSPs), nuclear reorganization-related proteins, TFs, and phytohormone signaling in rice resistance, whereby proteins related to this pathogen perception and signal transduction are important during the early stage of infection (Vo et al., 2021). A recent study using iTRAQ found that probenazole-inducible protein 1 (PBZ1) and phenylpropanoid accumulated in both resistant and susceptible cultivars. *Rhizoctonia solani* is a necrotrophic fungus that causes sheath blight in rice. ROS, SA, JA, aromatic aliphatic amino acids, and phenylpropanoid intermediates also accumulated, accompanied by the suppression of myo-inositol, indicating the loss of antioxidant activity, which is consistent with the formation of lesions by necrotrophic pathogens. *Cochliobolusmiya beanus*, which causes brown spot disease in rice, suggested a pattern similar to that seen in response to *R. solani* infection. Proteins involved in the Calvin cycle (fructose bisphosphate aldolase, sedoheptulose-1, 7-bisphosphatase, and RuBisCO) were reduced; however, oxaloacetate aspartate and aminotransferase, which are required for amino acid biosynthesis, and enzymes involved in redox homeostasis (peroxiredoxins, glutathione reductase, and NADP-dependent isocitrate dehydrogenase) were found to be accumulated (Vo et al., 2021). Proteomics studies

on rice response to BPH infection have revealed the occurrence of dynamic changes. Lipid transport and metabolism, SM biosynthesis, amino acid transport and metabolism, and phytohormone signaling are commonly induced by BPH in both susceptible and resistant cultivars (Zha and You, 2020).

9.2 Abiotic stresses

9.2.1 Drought

Osmotic stress induces a series of molecular and physiological responses, including stomatal closure, osmotic adjustment, reduced photosynthetic activity, and abscisic acid (ABA) synthesis. Decreased water loss due to stomatal closure and/or reduced stomatal density is an example of a drought-avoidance mechanism (Hadiarto and Tran 2010). However, a consequence of this strategy often reduces photosynthesis and growth because of a decrease in carbon uptake. A rice lectin protein, *Osr40c1*, plays a crucial role in imparting drought stress tolerance in plants by modulating *OsSAM2* as well as the transcriptional regulators *OsSAP8*, *OsMNB1Bn* and *OsH4* (Sahid et al., 2020). Drought-responsive proteins in rice revealed the photosynthesis-related adaptations to drought stress,a major hub gene; *LOC_Os04g38600* (encoding a glyceraldehyde-3-phosphate dehydrogenase), suggesting that photosynthetic adaptation via NADP(H) homeostasis contributes to drought tolerance in rice (Chintakovid et al., 2017). The drought-responsive proteins in vegetative tissues are unique to individual genotypes, suggesting diversity in the metabolic responses to drought. A total of 213 proteins were identified in a single genotype, 83 of which were increased in abundance in response to drought stress (Hamzelou et al., 2019).

9.2.2 Salinity

Salinity affects all stages of growth and development of rice, particularly early seedling, pollination, and fertilization stages (Saleethong et al., 2016). In tolerant rice cultivar like Pokkali more responsiveness of the proteins relating to photosynthesis light reactions, energy metabolism, antioxidant enzymes in the leaves, and stress proteins with protective roles in the grains compared to sensitive rice cultivar (Saleethong et al., 2016). A higher abundance of proteins involved in photosynthesis (such as oxygen-evolving enhancer proteins *OEE1,OEE3,* and *PsbP*) and stress tolerance (such as ascorbate peroxidase, superoxide dismutase, peptidyl-prolyl *cis-trans* isomerases, and glyoxalase II), were observed in shoots of tolerant genotype compared to sensitive genotype (Lakra et al., 2019). In response to salinity, selected proteins such as ribulose bisphosphate carboxylase/ oxygenase activase, remained elevated in Pokkali shoots. Glutamate dehydrogenase - an enzyme that serves as an important link between the Krebs cycle and metabolism of amino acids was found to be highly induced in Pokkali in response to stress. Similarly, other enzymes such as peroxidases and triose-

phosphate isomerase (TPI) were also altered in roots in response to the stress (Lakra et al., 2019).

9.2.3 High and low temperatures

Heat tolerance is conferred by maintaining plant functions and efficient scavenging of reactive oxygen species and stabilizing the structure and function of protein and enzymes by heat shock proteins (HSPs) (Maestri et al., 2002). Besides, heat-shock protein, a number of protection proteins was up-regulated under high-temperature stress. Proteomic analysis of rice seedlings showed upregulation of isoforms of glyoxalase I and a salt-induced protein under various high temperature stresses (35, 40 and 45^{0}C). Recent proteomic analyses revealed dynamic changes of metabolisms during rice grain development. Interestingly, proteins involved in glycolysis, citric acid cycle, lipid metabolism, and proteolysis were accumulated at higher levels in mature grain than those of developing stages. High temperature (HT) stress during the rice ripening period causes damaged (chalky) grains for loose packing of round shape starch granules (Mitsui et al., 2013).A total of 35 unique differentially abundant proteins, including a basic helix-loop-helix transcription factor (bHLH96), calmodulin-binding transcription activator, heat shock protein (Hsp70), and chaperonin 60 (CPN60) were detected in the proteomic analysis of II8 (II YOU 838) hybrid rice cultivars under heat stress (Wang et al., 2020b).

Cold stress also affects the rice protein expression to cope up with the stress. Functional analysis revealed differential expression of proteins involved in transport, photosynthesis, generation of precursor metabolites and energy; and, more specifically, histones and vitamin B biosynthetic proteins were observed to be affected by cold stress (Neilson et al., 2011). Many studies have identified many common molecular responses to low temperature including proteins involved in reactive oxygen species (ROS) detoxification, photosynthesis, and translation (Neilson et al., 2011).A total of 366 unique proteins involved in ATP synthesis, photosystem, reactive oxygen species, stress response, cell growth, and integrity were identified in response to cold stress in DC907 (cold-resistant hybrid wild rice). While both DC907 and 93–11(cold-sensitive cultivar) underwent similar alterations in proteomic profiles in response to cold stress, DC907 responded in a prompter manner in terms of expressing cold-responding proteins, maintained a higher level of photosynthesis to power the cells, and possessed a stable and higher level of DIR proteins to prevent the plant from obtaining irreversible cell structure damage (Wang et al., 2018c).

9.2.4 Yield related traits

The yield potential of rice basically based on the amount of filled grain per panicle, and hence poor grain filling plays a major role to diminish it. The compact panicle

rice varieties with high grain numbers per panicle shows poor grain filling or unfilled grain in inferior spikelets compared to lax panicled cultivar (Sekhar et al., 2015). The proteomic studies revealed that the superior and inferior spikelets of Mahalaxmi differentially expressed 21 proteins that were also expressed in Upahar. The protein analysis showed two differentially expressed proteins between the superior and inferior spikelets, in Mahalaxmi (compact panicle)and Upahar (lax panicle).The metabolic activities of the spikelets occupying the superior and inferior positions on the panicle were very different in Mahalaxmi compared with those in Upahar (Das et al., 2016).Among the proteins that were down-regulated in the inferior spikelets compared with the superior ones in Mahalaxmi were importin-á, elongation factor 1-â, and cell division control protein 48, which are essential for cell cycle progression and cell division. Low expression of these proteins might inhibit endosperm cell division in the inferior spikelets, limiting their sink capacity and leading to poor grain filling compared to that in the superior spikelets.

10. ROLE OF METABOLOMICS IN BIOTIC & ABIOTIC STRESSES

Metabolomics study includes organic acids, peptides, secondary metabolites, steroids, hormones, ketones, vitamins, aldehydes, amino acids, and lipids to generate extensive data. In rice, metabolomics techniques are thought to be not only useful to understand the biological traits of rice, such as the yield and defense responses, but are also helpful to improve rice quality, including its taste and nutritive value (Fernie et al., 2006). Metabolomics studies of the floral organs showed sugar starvation under drought and heat stress which resulted in reproductive failure in the grain in rice (Li et al., 2015). Recently, it has been found that metabolomics methods help to generate new rice cultivars in the context of identification of valuable gene functions and safety assessment of GM crops (Yonekura-Sakakibara et al., 2006). Several techniques or method shave been developed to determine metabolomes. In metabolomics, no single technique or tool can be used to analyze all the metabolites present in a metabolome; instead, a set of different technologies are required to provide the greatest amount of metabolite coverage. Different metabolomics techniques include mass spectrometry (MS), non-destructive nuclear magnetic resonance spectroscopy (NMR), high-performance thin-layer chromatography (HPTLC), capillary electrophoresis-mass spectrometry (CE-MS), gas chromatography-mass spectrometry (GC-MS), liquid chromatography-mass spectrometry (LC-MS), direct infusion mass spectrometry (DIMS), ultra-performance liquid chromatography (UPLC), high-resolution mass spectrometry (HRMS), and Fourier transform ion cyclotron resonance mass spectrometry (FI-ICR-MS). Out of these, CE-MS, LC-MS, GC-MS, and NMR-based integrated approaches have been extensively applied for metabolomics analysis (Razzaq et al., 2019).

10.1 Biotic stresses

Metabolomics study revealed differences between resistant and susceptible plants against *Xanthomonas oryzae pv. oryzae (Xoo)* in the accumulation of metabolites before and after infection. The resistant plants carrying *Xa21* contained more responsive metabolites, including rutin, pigments, fatty acids and lipids, and arginine, which were required for polyamine biosynthesis and alkaloid metabolism, and virulence signal acetophenone depression in *Xa21* (Vo et al., 2021). Changes in phenolic compounds, such as flavonoids and hydroxycinnamic derivatives were found to differ depending on the cultivar-plant growth-promoting rhizobacteria (PGPR) strain interaction. *Pseudomonas aeruginosa* was found to produce compounds associated with systemic acquired resistance (SAR), including siderophores (1-hydroxy-phenazine, pyocyanin, and pyochellin), and antibacterial compounds, including 4-hydroxy-2-alkylquinolines and rhamnolipids. *Magnaporthe oryzae*, the causal agent of rice blast disease help to prevent fungal invasion *via* the antimicrobial activity of phytoalexins, including N-benzoyl tryptamine, N-cinnamoyl tryptamine, sakuranetin, and phenylamides, or the ROS-scavenging activity of serotonin. Interestingly, a metabolomic assay using HPLC identified a rice saponin, Bayogenin 3-O-cellobioside, which is the first saponin found in rice is well correlated with blast resistance (Vo et al., 2021). A metabolomics study in leaf sheath revealed enhanced fatty acid oxidation, glyoxylate cycle, gluconeogenesis, and ã-aminobutyric acid (GABA) shunt in susceptible cultivars, whereas glycolysis was up-regulated in resistant cultivars, resulting in the production of substrates for SM synthesis *via* the shikimate pathway after BPH infection. Further, amino acids, organic acids, and fatty acids were also found to be stable in resistant cultivars. Higher levels of flavonoid glycosides (schaftoside, iso-schaftoside, rhoifolin, and apigenin 6-C-á-l-arabinoside-8-C-â-l-arabinoside) were induced in resistant rice compared with susceptible rice (Vo et al., 2021).

10.2 Abiotic stresses

10.2.1 Drought

Plants respond to drought by reprogramming their transcriptional, proteomic, and metabolic pathways in order to protect cells from stress-mediated damage. Many studies have shown that the levels of glucose, fructose, and the raffinose family oligosaccharides (RFOs) increased in plants under drought compared with control conditions (Krasensky and Jonak, 2012). Further ascorbate and dehydroascorbate levels were increased in response to drought and an increase in ascorbate and dehydroascorbate occurred as early as the increase in RFOs (Fàbregas and Fernie, 2019). γ-aminobutyric acid (GABA), proline, valine, leucine, isoleucine, threonine, lysine, and â-alanine amino acid levels were only significant

under severe drought stress, whereas sugars accumulated under mild drought stress conditions in rice plants (Fàbregas and Fernie 2019). Further aromatic amino acids (AAAs) serve as precursors for a large number of secondary metabolites and are known to increase under drought conditions (Vogt 2010). Rice showed decreased levels of citrate, oxoglutarate, succinate, and fumarate except for malate which increased under drought stress (Todaka et al., 2017). Anthocyanin and flavonol levels increased in response to drought (Nakabayashi et al., 2014).

10.2.2 Salinity

Compared with the effects of ion toxicity, rice is reported to suffer greater osmotic stress under saline conditions. The contents of several metabolites, including amino acids (AAs), sugars, and polyols, are increased under saline conditions, thereby serving as a defense against osmotic challenge by acting as compatible solutes (Wu et al., 2013). In the leaf of rice seedling of salt-tolerant rice cultivars, there was increased production of the 2 signaling molecules, serotonin and gentisic acid for important biomarker compounds (Gupta and De 2017). The fluxes of tyrosine amino transferease, arogenate dehydrogenase (ADH), and L-arogenate: oxaloacetate aminotransferase (aka prephenate aminotransferease) reactions were enhanced under salt stress compared to control condition suggesting an increase in aromatic amino acid metabolism of salinity-induced rice (Wanichthanarak et al., 2020).

10.2.3 High and low temperatures

Rice is susceptible to heat stress particularly during the reproductive stage of the crop. Reduced grain yield and quality were associated with high-temperature stress, and specifically with high night-time temperatures (Dhatt et al., 2019).Metabolite profiles during early seed development (3 and 4 days after fertilization (DAF) were distinct from the lategrain filling stages (7 and 10 DAF) under optimal conditions and observed that accumulation of sugars (sucrose, fructose, and glucose) peaked at 7 DAF suggesting a major flux of carbon into glycolysis, tricarboxylic acid cycle, and starch biosynthesis. The most prominent changes were exhibited by the differential abundance of sugar and sugar alcohols under during the grain filling stage under high-temperature stress (Dhatt et al., 2019). Amino acids, citric acid cycle intermediates, and many metabolites of carbohydrate metabolism were affected in response to high and low temperatures. It was concluded that at high temperatures, proline or its biosynthetic/degradation intermediate, pyrroline 5 carboxylate are toxic, and proline is therefore not a suitable osmolyte/compatible solute. Glutamine was specifically elevated in plants subjected to a combination of drought and heat stress, suggesting that when proline biosynthesis was inhibited, glutamate was re-channeled into glutamine instead (Guy et al., 2008).Rice plant is also sensitive to low-temperature (LT) stress.

Seven key metabolites (N-acetyl-L-glutamic acid, L-ascorbate, anthranilic acid, inositol, Lysine butyrate, and N-caffeoyl putrescine) were identified, which were responded significantly to LT conditions and promoted seedling growth to under LT (Yang et al., 2019b).

11. CONCLUSION AND FUTURE THRUST

Genetic improvement beyond current capabilities is needed to meet the growing world demand in rice not only for food but also for high quality produced from less land, water, other inputs, while at the same time conserving soil and genetic resources. Omics approaches are overlapping and inter-dependent. The integration of all data obtained from omics approaches is vital to conclude and comprehend major cell response-cascade, which may differ among tolerant and sensitive rice varieties during biotic and abiotic stresses. Several QTL/ genes have been identified, cloned, and functionally characterized at the molecular level. Many QTL/ genes have also been utilized in molecular breeding programs, and several varieties with desired traits have been developed. The knowledge on genes and genomes, germplasms, and genomic data obtained through omics studies are sufficient to change strategies and technologies in rice breeding, which can be termed as ''designed genomic breeding.'' Despite the tremendous progress that has been made by omics approaches, there is still a huge gap of knowledge between genotype and phenotype. It is essential for breeding high-yielding climate-resilient rice varieties with better grain qualities, input use efficiency, plant architecture, C4-ness traits, nitrogen-fixing ability, and apomicts. We have little success in C4ness, nitrogen-fixing ability, apomix, input use efficiency, and yellow stem borer resistance. We need to put more efforts into these areas, and also carry whole system approach research works to understand gene interactions, and utilize them in breeding programs.

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