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Multi-trait improvement in rice through marker-assisted breeding

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ABSTRACT

Biotechnological tools like molecular markers can add precision to breeding and accelerate breeding efforts. Towards this objective, our research team at ICAR-IIRR has applied marker-assisted breeding (MAB) for improvement of multiple traits like resistance against bacterial blight, blast, gall midge and BPH, heterosis related traits, improvement of low soil P tolerance, grain quality and yield. Through marker-assisted backcross breeding (MABB), a high-yielding, bacterial blight resistant rice variety possessing fine-grain type and low glycemic index (GI), named Improved Samba Mahsuri (ISM) has been developed and released for cultivation by farmers. MABB has also been applied for improving bacterial blight resistance of a few important traditional and evolved Basmati rice varieties and hybrid rice parental lines. A novel bacterial blight resistance gene, Xa33 has been identified from an accession of the wild rice, O. nivara, fine-mapped and transferred into several elite genetic backgrounds. Novel sources of resistance against bacterial blight and blast diseases have been identified and characterized and major blast resistance have been transferred to several elite genetic backgrounds and a major QTL associated with neck blast resistance has been identified from wild rice. Additionally, gene-pyramid lines possessing resistance against gall midge have also been developed and a novel and highly effective BPH resistance gene has also been identified and mapped with molecular marker and few promising donors possessing resistance against sheath blight have been identified. A molecular marker-based assay has been designed for rapid and accurate determination of impurities in seed-lots of rice hybrids and their parental lines and functional markers have been developed for the traits relevant to hybrid rice, viz., wide-compatibility, wild-abortive cytoplasmic male sterility and fertility restoration. Functional markers have also been developed for major grain quality determining genes, fgr and GS3 and a major QTL controlling gelatinization temperature has been identified through molecular mapping. The major QTL responsible for low soil phosphorus (P tolerance, viz., Pup1 has been transferred to Improved Samba Mahsuri, MTU1010 and IR64 and novel, non-Pup1 type donors have been identified for the trait. Four major yield enhancing genes, viz., Gn1a, SCM2, OsSPL14 and GW5 have been transferred to elite rice cultivars, viz., Improved Samba Mahsuri, Swarna, MTU1010 and NDR359.

Key words: *Rice, molecular markers, biotic stress resistance, heterosis breeding, low soil phosphorus tolerance, grain quality, yield*

INTRODUCTION

Rice is the world's most important food crop and more than 90% of the world's rice is produced and consumed in Asia, where 60% of the people live. In India, rice feeds more than 70% of the population and is the principal calorie source for most of the people. In the last six decades, rice production has steadily kept in pace with the population growth rate in India and elsewhere, mainly due to the gains derived from the technologies of green revolution era and due to the ushering of new technologies like hybrid rice. In the last decade, rice yield levels have reached plateau and no significant increase is being witnessed in productivity levels in the last few years (Shobha Rani et al., 2013). Keeping in view the annual average population growth rate of ~1.5% and estimated per capita consumption of about 250 g of rice per day, the demand for rice is

anticipated to be at least 140 M tonnes by 2025 (http://www.fao.org/rice2004/en/pdf/khush.pdf).This projected demand can be met only if there is a steady increase in productivity and production. Further, the increases in production have to be achieved under conditions of declining and deteriorating land, soil and water resources and a rapidly changing climate. Deployment of conventional tools and techniques can be helpful only to a limited extent to address these daunting challenges. Modern tools of biotechnology can be helpful to meet the rice production and productivity targets. Among the biotechnology tools of importance to rice breeding, marker-assisted selection can be considered as the most important as it can accelerate breeding efforts tremendously (Sundaram et al., 2013).

Considering the imminent need to increase rice production and productivity significantly, in the coming decades, molecular breeding has been deployed by our research team as a tool for the last two decades at ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, India for improvement of various traits of agronomic importance, for which sufficient genetic variation is available in rice. These include resistance against biotic stresses like bacterial blight (BB), blast, rice gall midge, brown plant hopper, heterosis breeding, enhancement of tolerance to low soil P, developing varieties with superior grain quality and yield etc.

Marker-assisted breeding for durable resistance/ tolerance against biotic stresses

Intensification of agriculture, particularly after the advent of high-yielding, fertilizer responsive rice varieties during the and post the green revolution era, the economic loss caused by insect pests and diseases has increased tremendously. In recent times, this challenge has acquired new dimensions of increased severity and unpredictability under the widespread scenario of climate change.

Insect pest control remains a core problem for Asian and Indian rice farmers. Yield losses of 25% or more have been attributed to "ravages due to pests" in rice (Oerke et al., 1994). The major insect pests of significance in rice are yellow stem borer (SB), brown planthopper (BPH), White backed plant hopper (WBPH), leaf folder, gall midge, green leaf hopper (GLH) and gundhi bug. From an Indian perspective, stem borers have accounted for 30% of the losses followed by planthoppers (20%), gall midge (15%), leaf folder (10%) and other pests (25%) (http://www.rkmp.co.in). Similar to insect pests, diseases caused by bacteria, fungi and viruses are also one of the key biotic stresses affecting rice crop is infected by many. The major ones are blast, bacterial blight, sheath blight, rice tungro disease and false smut. Bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* and rice blast caused by *Magnaporthe oryzae* continue to be major production constraints in rice cultivation. In recent years, these diseases have been reported regularly at an alarming intensity in areas earlier considered non-endemic to these diseases.

Though novel and effective chemicals have been developed to combat the pests and diseases, their indiscriminate and injudicious use has resulted in the development of pesticide/fungicide resistant strains of pests and pathogens making chemical control, less effective and also leading accumulation of harmful residues and wide scale environmental pollution. Deployment of resistant cultivars is potentially is the most viable, cheap and environmentally friendly option for the farmers to combat the pests and diseases. Major diseases in rice such as bacterial blight and blast and pests like BPH and gall midge can be managed through deployment of resistant cultivars. However, efforts to breed for resistance to biotic stresses, especially bacterial blight and blast have often been thwarted by appearance of newer and more virulent forms of these pathogens, especially when resistance is based on single gene. Under these circumstances, conventional breeding strengthened by molecular tools such as marker aided selection and gene pyramiding with the help of molecular markers appears to be the most promising approach, for evolving a broad spectrum and durable resistance mechanism to most of the biotic stresses.

Marker-assisted breeding for durable disease resistance

Among the major diseases of rice, bacterial blight and blast are two of the most important ones, with sheath blight and RTV being the next most important diseases. In the recent years, incidence of disease like false smut, Bakanae and stem rot is also increasing. Among the diseases, sufficient genetic variation is available for resistance against bacterial blight and blast in rice.

Hence, we focused on developing resistance against the two diseases in rice.

Bacterial blight (BB) resistance

Deployment of varieties carrying one or more major resistant genes is the most effective approach for managing the disease. Resistance to BB is generally considered qualitative in nature and there are only a few reports about quantitative resistance to the disease (Nino-Liu et al., 2006). Till date, at least 40 BB resistance genes have been identified from diverse sources (Kim et al., 2016). The resistance genes have been designated as *Xa1* to *Xa40* with eight of them being recessive (*xa5, xa8, xa13, xa15, xa19, xa20, xa24* and *xa2*). Six genes have been cloned (*Xa1, xa5, xa13, Xa21, Xa3/Xa26* and *Xa27*) and at least another six have been physically fine-mapped (*Xa2, Xa4, Xa7, Xa30, Xa33* and *Xa38*). Even though majority of these genes have been identified from the cultivated rice, i.e. Oryza sativa, some have been introgressed from related wild species like *O. longistaminata* (*Xa21*), *O. rufipogon* (*Xa23* & *Xa34t*), O. minuta (*Xa27* & *Xa35t*), *O. officinalis* (*Xa29t*), *O. australiensis* (*Xa32t*) and *O. nivara* (*Xa33* & *Xa38*). Donors possessing some of these genes like Xa4, xa5, xa13 have been used widely in classical/conventional rice breeding programs. Most of the resistance genes are dominant in nature (e.g., *Xa4, Xa7, Xa21* etc.), while some are recessive (e.g., *xa8, xa13*) and some display semi-dominance (e.g., *Xa5, Xa27*). The genetics of resistance to bacterial leaf blight is well studied and the individual resistance genes are available in a common varietal (IR24) background as near isogenic lines (NILs) (Ogawa et al.,1988). Some of the 'R' genes are effective only in adult plants (e.g., *Xa21*) while others do not seem to be developmentally regulated. It is now generally accepted that pyramiding two or more effective resistance genes is the way forward for broadening the spectrum and also the durability of resistance. Among the different genes available, *xa5, xa8, xa13, Xa21, Xa33* and *Xa38* are known to be effective under Indian conditions.

Through marker-assisted backcross breeding, the highly-popular and prized rice variety, Samba Mahsuri has been introgressed with three major BB resistance genes, *Xa21, xa13* and *xa5* and a new variety- Improved Samba Mahsuri (ISM) has been developed and released for cultivation by Indian farmers (Sundaram et al., 2008a). Under bacterial blight infection, Improved Samba Mahsuri (ISM) gives ~ 25-40% more yield than the susceptible varieties like Samba Mahsuri, HMT sona, PKV HMT etc. Improved Samba Mahsuri fetches premium price like Samba Mahsuri and other elite fine-grain type rice varieties. Hence, the new variety is increasingly getting popular in bacterial blight endemic areas throughout the country ISM is estimated to be cultivated in a cumulative area of > 1,50,000 ha (Reddy, 2017). Recently, a study done by ICAR-National Institute of Nutrition has established that ISM has a very low GI value of 50.99, making it suitable for consumption by those who have diabetes. Towards adding value to ISM, genes conferring resistance against blast (*Pi2* + *Pi54*), tolerance to low soil P (*Pup1*) have been transferred to the bacterial blight resistant variety and breeding lines are under evaluation under AICRIP. Our team is presently transferring genes conferring resistance against BPH (*Bph33*), gall midge (*Gm4* +*Gm8*) along with tolerance to low soil P (*Pup1*) and salinity (Saltol) into ISM through marker-assisted backcross breeding. We have also transferred the three BB resistance genes into the genetic background of two traditional Basmati varieties, Taraori and Basmati 386 (Pandey et al., 2013) and into few evolved Basmati varieties, Vallabh Basmati 22, IET18006, Vasumati and Sugandhamati (Srikanth et al., 2016), Triguna, a pest resistant variety (Sundaram et al., 2009) and transferred the dominant gene, *Xa21* into the hybrid rice parental lines KMR3R (Hari et al., 2011) and IR58025B (Hari et al., 2013).

Existence of different pathotypes of the bacterial blight pathogen in India has been reported by different research groups (Yashitola et al., 1997; Mishra et al., 2013) and many single gene containing rice varieties are rapidly becoming susceptible to the pathogen. Through a recent study involving pathotyping analysis coupled with genotyping using the bacterial pathogen genome specific probes, our research team has established the existence of 22 pathotypes of the pathogen across India (Yugandhar et al., 2017). This necessitates identification of new sources of resistance and their molecular mapping so that the resistance can be broad-spectrum and durable. Towards this objective, we have identified a novel dominant resistance gene, named *Xa33* from an accession of the wild rice, *O.*

nivara, fine mapped it on Chr. 7 and transferred it to Samba Mahsuri with the help of markers (Natarajkumar et al., 2012). A recessive gene locus controlling resistance was identified from an accession of *O. rufipogon* and it was mapped on Chr. 1 (Natarajkumar et al., 2011). We have also identified a major recessive gene locus on Chr. 8 from the resistant check variety, Ajaya (Sujatha et al. 2011). We have also identified a novel BB resistance gene, *Xa33* from an accession of *O. nivara*, fine-mapped it on Chr. 7 of rice and introgressed it into Samba Mahsuri, ISM, Akshaydhan, RPHR1005R and DRR17B along with *Xa21*. Recently, through screening of multiple isolates of the bacterial blight pathogen, we have identified that two introgression lines of O. officinalis, *viz.,* IR75084-15-3-B-B and IR75084-74-8-B-B-B possess novel, monogenic, dominant resistance against BB and mapping the gene(s).

Blast resistance

Till date, more than 100 blast resistance genes and more than 350 quantitative trait loci (QTLs) have been detected (http://www.oryzabase.com). It is interesting to note that genes affecting blast resistance are co-localized on chromosomes 6, 11 and 12. On chromosome 6, at least 14 genes and/or alleles (*Pi2, Piz, Piz-t, Piz-5, Pi8(t), Pi9, Pi13, Pi13(t), Pi25(t), Pi26(t), Pi27(t), Pid2, Pigm(t)*, and *Pi40(t)* have been mapped in the region near the centromere. On the long arm of chromosome 11, at least nine genes (*Pi1, Pi7, Pi18, Pif, Pi34, Pi38, Pi44 (t), PBR*, and *Pilm2*) and six alleles at the Pik locus (*Pik, Pik-s, Pik-p, Pik-m, Pik-h*, and *Pik-g*) have been mapped. On chromosome 12, at least 17 resistance genes and/or alleles *Pita, Pita-2*, *Pitq6, Pi6(t), Pi12(t), Pi12(t), Pi19(t), Pi20(t), Pi21(t), Pi24(t), Pi31(t), Pi32(t), Pi39(t), Pi62(t), Pi157(t) IPi*, and *IPi3*) have been mapped in the region near the centromere. For Indian conditions, the blast resistance genes, *Pi1, Pi2, Pi5, Pi9, Pi40, Pi54, Pi-ta, Pizt* etc. are known to be effective.

Utilizing some of the effective resistance genes mentioned above, we have development gene-pyramid lines possessing resistance against blast and also developed multiple resistant trait lines. Madhavi et al. (2012) stacked the blast resistance gene, *Pi54* into the genetic background of the elite fine-grain type, high yielding, bacterial blight rice variety; Improved Samba Mahsuri through MAS. Hari et al. (2013) introgressed the blast resistance gene Pi54 and a bacterial blight resistance gene *Xa21* into the genetic background of the elite maintainer line IR58025B with the help of markers. Recently, Madhavi et al. (2016) has transferred also transferred two blast resistance genes, *viz., Pi2* and *Pi54* into Improved Samba Mahsuri. Balachiranjeevi et al. (2015) has transferred the two blast resistance genes into the background an elite maintainer line, DRR17B along with the major bacterial blight resistance gene, *Xa21*. Abhilash Kumar et al. (2016) has transferred the same set of bacterial blight and blast resistance genes into RPHR1005R, the male parent of the popular rice hybrid, DRRH3. In recent years, neck blast diseases is assuming significant importance and in order to address this problem, Recently, a major QTL conferring high level of tolerance to neck blast has been identified on Chr. 3 from an introgression line of *O. glumaepatula* and the same has been transferred into the genetic background of several elite rice varieties (Aglawe et al., 2017). Through a recent study, we have also identified a few introgression lines of *O. glaberrima, O. longistaminata, O. latifolia* and *O. minuta* which possess good resistance against blast disease.

Sheath blight tolerance

Tolerance to sheath blight has been an elusive target in rice breeding programmes and not much progress has been witnessed in this regarding for the last several years. We have identified the rice varieties/genotypes *viz.,* SM 801 (N 22 mutant), 10-3 (Introgression line), Ngnololasha, Wazuhophek, Gumdhan and Phougak (land races from north east) and RP 2068-18-3-5 are tolerant to sheath blight. It is interesting to note that none of the above mentioned lines possess any known QTLs for tolerance (like those from Tetep, Teqing, Jasmine 85 etc., as inferred through analysis with linked markers). Among the tolerant lines identified, Wazuhophek was observed to show a high level of tolerance against the pathogen (Badri et al., 2016) and we have developed a RIL mapping population from the cross Wazuhophek/Improved Samba Mahsuri (highly susceptible to sheath blight) and are presently undertaking molecular mapping. Using a RIL mapping population derived from the cross, RP2068-18-3-5/TN1, we have identified a QTL explaining 7.8 % of

phenotypic variance located on Chr. 5.

Marker-assisted breeding for durable insect pest resistance

Even though genetic variation is still elusive in rice for important pests like stem borer and leaf folder in rice, sufficient genetic variation is available for two other major pests, *viz.,* rice gall midge and brown plant hopper (Sundaram et al., 2014).

Gall midge resistance

Rice varieties differ in their response to gall midge infestation. A small proportion of varieties are immune to the pest attack by effectively killing the maggot within hours of feeding. The antibiosis mechanism displayed by varieties is distinctly of two different types. Majority of such varieties express hypersensitive reaction (HR) leading to tissue necrosis at the site of maggot feeding and are referred to as HR+ve type while few of the resistant genotypes that do not display HR but still maggot mortality is seen are termed as HR-ve types. Nature of resistance being antibiosis, the host plant resistance is the most effective way of managing the pest (Bentur et al., 2003). Field and greenhouse evaluation of more than 50,000 germplasm accessions has resulted in identification of over 300 primary sources of resistance. Using some of these sources of resistance, over 155 gall midge resistant rice varieties have been developed and released for commercial cultivation since 1972 (Shobha Rani et al., 2011). However, rapid evolution of virulent biotypes of the pest capable of overcoming plant resistance is the current problem. Studies on the genetics of gall midge resistance in rice have shown involvement of, often, a single dominant or a recessive gene. Till date, eleven genes conferring resistance against the pest have been reported (Himabindu et al., 2010), ten of which are dominant (*Gm1* through *Gm11*, except *gm3*), and one (*gm3*) is recessive. Of the eleven gall midge resistance genes that are identified so far, nine genes (*Gm1, Gm2, gm3, Gm4, Gm5, Gm6, Gm7, Gm8* & *Gm11*) have been tagged and mapped (Sundaram et al. 2014). Presence of gall midge biotypes within the country was suspected during the early phase of breeding for resistance. So far, seven distinct biotypes have been characterized based on reaction pattern against five groups of differential rice varieties. (Vijaya Lakshmi et al., 2006). With the identification of molecular markers closely linked to most of the gall midge resistance genes, it is now easy to develop gene pyramids for wide spectrum and durable resistance. The crucial question being addressed now is which gene combinations meet these twin objectives. It has been suggested that deployment of two or more non-deployed genes that differ in their mechanism of resistance is the ideal option. Earlier studies (Bentur and Kalode, 1996; Bentur et al., 2003) have reported two distinct mechanisms of resistance in rice against gall midge. One of these involves expression of Hyper Sensitive Reaction (HR) + type and the other does not involve HR (HR- type). Only two of the genes (*Gm1* and *Gm8*) confer HR-ve type resistance. Hence a combination of *Gm4* or *gm3* (HR+) and *Gm1* or *Gm8* (HR-) genes would meet the above specified requirements. With this objective, we have attempted to pyramid resistance genes with differing mechanism of resistance. Through a research project sponsored by ICAR, we have successfully pyramided major resistance genes, gm3 and Gm8 into the genetic background of the elite, bacterial blight resistant rice variety, Improved Samba Mahsuri (Sama et al., 2012; Sama et al., 2013) through marker-assisted breeding (MAB). Our research team has also pyramided the genes, *Gm1* and *Gm4* into the genetic background of Improved Samba Mahsuri (Divya et al., 2015). Recently we have transferred Gm4 and Gm8 into the genetic background of RPHR1005R, the restorer line of the elite rice hybrid, DRRH3 (Abhilash Kumar et al., 2017). In addition, we have also identified that two breeding lines possessing introgression from O. glaberrima, *viz.,* IR 75870-5-8-5-B-2-B and IR 75870-5-8-5-B-1-B, received from IRRI, Philippines possess novel resistance against gall midge through repeated screening experiments coupled with marker-based allelism tests.

Brown planthopper (BPH) resistance

Several diverse donor sources of BPH resistance were identified from field and greenhouse screening in India as well as other countries. Host-plant resistance was characterized to be both qualitative and quantitative in inheritance, depending on the source of germplasm. The genetics of BPH resistance revealed presence of 32 major genes (*Bph1* to *Bp32*) in cultivated rice (*O. sativa*) and seven wild relatives, *Oryza australiensis*,

Oryza eichingeri, Oryza latifolia, Oryza officinalis Oryza minuta, Oryza rufipogon and Oryza glaberrima using classical genetics and molecular approaches. Of the 32 BPH resistance genes characterized so far in rice, nine are recessive in their inheritance (*bph2, bph4, bph5, bph7, bph8, bph12, bph16, bph19* and *bph24*. It is known that the resistance genes, *Bph3, Bph10, Bph13, Bph20* and *Bhp21* may be useful for India. Through a recent study, we identified that the rice breeding line RP2068-18-3-5 (originally derived from the cross Velluthacheera x Swarnadhan; Sarao and Bentur, 2016) shows good level of resistance against BPH and established that a major locus located on Chr. 1 controls resistance against the pest through analysis with SSR markers and novel gene controlling resistance has been named as *Bph33t*. Utilizing flanking SSR markers, we are in the process of transferring *Bph33t* into the popular variety, Improved Samba Mahsuri.

Application of molecular markers in heterosis breeding

In order to meet the rice production requirements of the future, adoption of hybrid rice technology is considered as one of the most feasible options. However, despite large-scale efforts done for the past several years, the area under hybrid rice is limited to about 3 Mha in 2016. This is due to several reasons like low magnitude of heterosis, not so desirable grain and cooking quality of the hybrids, susceptibility of rice hybrids to pests and diseases, low seed production rates (resulting in high cost of seeds), genetically impure seeds. While hybrid rice breeders are attempting to solve these problems to a significant extent through various strategies and in the recent years, new hybrids with enhanced grain yield heterosis and better grain quality have been developed, the application of molecular markers can accelerate heterosis breeding efforts and can help in good quality control in hybrid rice seed production. Towards this objective, we have utilized molecular markers for introgressed genes conferring resistance against a few major biotic stresses, developed protocols for rapid and reliable assessment of genetic purity of seeds of parental lines and hybrids, identified the molecular basis of traits like wild-abortive cytoplasmic male sterility, fertility restoration and wide compatibility and also identified a few markers suitable for heterosis prediction in rice.

Development of biotic stress resistant rice hybrids

One of the major limitations of the first and second generation India rice hybrids is their high level of susceptibility to diseases like bacterial blight and blast. In order to address this problem, Hari et al. (2011) has transferred the major dominant bacterial blight resistance gene, *Xa21* into an elite, stable restorer line KMR3R (restorer line for the elite rice hybrid, KRH2). Hari et al. (2013) have also improved the maintainer line of KRH2, *i.e.,* IR58025B for resistance against bacterial blight (*Xa21*), blast (*Pi54*) through marker-assisted selection (MAS). In this effort, we also did negative selection for aroma trait (targeting the candidate gene for aroma trait, badh2) and low amylose content (targeting the waxy locus) through MAS and developed breeding lines of the elite maintainer parent, which are devoid of aroma (desired by consumers in Southern part of India) and have high amylose content (and hence less sticky after cooking). Presently, we are in the process of converting the elite improved versions of IR58025B into WA-CMS through marker-assisted backcross breeding. Through a R & D project sponsored by the Department of Biotechnology, Government of India, we have also improved an elite maintainer line, DRR17B and two restorer lines, viz., RPHR1005R (which is the restorer line of the high-yielding hybrid, DRRH3, which possess the highly desirable MS grain type) and Akshayadhan for their resistance against bacterial blight (*Xa21* + *Xa33*), blast (*Pi2* + *Pi54*) and gall midge (*Gm4* + *Gm8*) [Balachiranjeevi et al., 2015; Abhilash Kumar et al., 2015; Bhaskar et al., 2015; Abhilash Kumar et al., 2016a; Abhilash Kumar et al., 2016b, Abhilash Kumar et al., 2017].

Marker-based assessment of genetic purity of seeds and molecular analysis of fertility restoration trait

Maintenance of genetic purity of seeds of parental lines and rice hybrids is vital for realizing the full potential of heterosis breeding in rice (Mao et al., 1996) and seed quality control is a vital part of any hybrid seed production unit. Conventionally, genetic purity of seeds is estimated through a morphological assay called 'Grow-out-test (GOT)', which has several limitations. As a replacement for GOT, we have developed a SSR

and STS marker-based assay for rapid and accurate determination of impurities in seed-lots of rice hybrids (Yashitola et al., 2002). This assay is single seed-based and utilizes parental polymorphic SSR markers for determination of impurities in hybrid seed-lots. We have also undertaken extensive fingerprinting of public bred rice hybrids and parental lines used in India and have identified genotype/hybrid-specific SSR markers for use in seed genetic purity assays (Sundaram et al., 2008b). In addition to maintenance of purity of hybrids, it is also essential to maintain purity of seeds of parental lines, particularly the WA-CMS line as any impurity creeping in the parental line multiplication process can magnify the level of impurities in the hybrid seed production process. We have identified a unique polymorphic sequence in the mitochondria of a WA-CMS line and targeting this sequence, we developed a dominant marker, which can distinguish WA-CMS lines from their cognate iso-nuclear maintainer lines and hence useful for assessment of impurities in seed-lots of WA-CMS lines (Yashitola et al., 2004). Later, a unique mitochondrial SSR sequence, which is polymorphic between WA-CMS lines and maintainer lines was identified and a co-dominant marker was developed. Using this marker, we have developed an assay for rapid and accurate determination of impurities in seed-lots of WA-CMS lines (Rajendrakumar et al., 2007). Recently, we did an extensive expression profiling of putative candidate mitochondrial genes, which have been earlier implicated with the trait of CMS in rice, validated and established the candidacy of gene, called WA352 (Pranathi et al., 2015). Targeting a deletion in the exonic part of WA352, a co-dominant marker, which can distinguish WA-CMS lines and maintainer lines has been developed and demonstrated for use in rpaid WA-CMS line seed purity assessment (Pranathi et al., 2016a). This assay and the assay for hybrid seed purity testing are being routinely deployed for analysis of impurities in seed-lots provided by both public and private sector companies at ICAR-IIRR. Recently, targeting the candidate genes for the major fertility restorer genes for WA-CMS in rice, viz. *Rf4* (PPR9) and *Rf3* (SF21S), we have developed functional markers, which can clearly distinguish restorer lines from non-restorer ones (Pranathi et al. 2016b). The study also identified that *Rf4* is the major loci explaining about 80-85 % of trait variance and *Rf3* plays a minor role (5-10 % of trait variance), with several minor QTLs playing a role.

Molecular markers for wide-compatibility genes

Even though pronounced heterosis in inter-subspecific hybrids (*i.e., Indica* x *Japonica* hybrids) has been known in rice for a long time, its exploitation for hybrid rice breeding has been hampered due to moderate to severe sterility, which is often noticed in such hybrids. For the last three decades, a few inter-subspecific hybrids have been developed by incorporating wide-compatibility genes that resolve hybrid sterility into parental lines of the inter-subspecific hybrids (Ikehashi and Araki, 1986). So far at least 21 such wide-compatibility genes have been identified in rice. For effective use of the trait in heterosis breeding, it is necessary to undertake molecular mapping of the genetic loci underlying the trait of wide-compatibility, so that molecular markers specific for the major wide-compatibility loci can be identified and utilized. Towards this objective, we undertook molecular mapping utilizing a three-way cross hybrid, identified that the wide compatibility loci, S5 and S8 located on Chr. 6 play a major role and developed SSR markers that flank both the loci (Singh et al., 2005). Later, we also under-took fine-mapping of S5, validated the candidate gene for the locus (*i.e.,* Aspartyl protease), developed a functional, co-dominant marker targeting the candidate gene and demonstrated the utility of this marker in accurate determination of allelic status at *S5* (Sundaram et al., 2010). This functional marker is now being extensively used in various rice breeding programmes aimed at development of superior inter-subspecific rice hybrids.

Marker-based prediction of heterosis

Prediction of parental combinations which can give highly heterotic hybrids has been a major objective of any hybrid rice breeding program. Empirical evidence indicates that the establishment of divergent maintainer and restorer groups is necessary for success of hybrid breeding programmes (Melchinger and Gumber, 1998). To increase hybrid breeding efficiency, diverse data sets, such as morphological traits, isozymes, storage protein profiles, pedigree records and DNA markers have been employed for assessing the parental genetic diversity, which was then correlated with grain yield heterosis. However none of these attempts could

conclusively establish a clear correlation between genetic divergence among the parents and yield heterosis. We attempted to assess the potential a special class of functional markers called EST-SSRs in predicting heterotic cross combinations. Through a study involving 60 hybrids, 50 each of hyper-variable genomic and EST-SSR markers, we have identified that a set of 15 EST-SSR markers, 25 hyper-varible genomic SSR markers and a set of 20 SSR markers targeting (GATA)n motifs can be useful for prediction of heterosis (Rajendrakumar et al., 2009; Jaikishen et al., 2010). Presently, we are studying the expression of selected rice hybrids and exploring the possibility of developing a SNP marker-tool set for use in heterosis prediction.

Marker-based analysis of key grain quality loci in rice

Improvement of grain quality has been the one of the most important objectives of rice breeding, after yield improvement. Grain quality, comprising of 16 physicochemical characteristics (Shobha Rani et al., 2011) is a complex trait in rice. Among the characteristics, amylose content, amylopectin content, amylose:amylopectin ratio, gelatinization temperature, gel consistency, alkali spreading value, kernel length, kernel breadth, length/breadth ratio, kernel length after cooking and aroma are the most important. After the sequencing of the rice genome, the genomic loci underlying these traits are now being discovered and it is known that a few key loci like waxy, granule bound starch synthase, soluble starch synthase, starch branching enzyme, badh2 etc. play a key role in controlling various traits associated with grain quality and markers linked to these loci have been developed earlier. In an association study involving 380 indica rice genotypes, we validated the reported molecular markers and established that the markers show varied levels of association for each trait (Shobha Rani et al., 2011). Three markers targeting starch branching enzymes showed association with amylose content and gelatinization temperature. Through analysis of a population segregating for the trait of gelatinization temperature, a major QTL, named qGT6, explaining > 30 % of phenotypic variance was discovered between the SSR marker interval RM276-RM216 on Chr. 6 (Sivaranjani et al., 2010). A functional marker, named BADEX7-2, targeting a 8-bp indel in the candidate gene for fragrance/aroma trait, *i.e.,* badh2 has been developed. This marker can unequivocally distinguish aromatic rice varieties from non-aromatic ones (Sakthivel et al., 2008). We have also developed a functional marker, named DRR GL01, for the traits kernel length and kernel length after cooking, targeting an indel in the candidate gene for grain size and shape, *viz.,* GS3 (Ramkumar et al., 2010). The validated markers are now routinely being used for breeding work in ICAR-IIRR, Hyderabad and other Institutes.

Breeding for low soil phosphorus tolerance

Phosphorus (P) is a vital element required for the growth and development of rice crop. Unfortunately, natural reserves of P are very limited, restricted to a few countries and they are entirely non-renewable and the continuing demand for P could deplete global P reserves by the end of the century (Byrne et al., 2011). This coupled with reduced subsidy and increasing cost of phosphatic fertilizers in major rice growing countries like India is resulting in significantly reduced application of such fertilizers in the recent years. A major part of rice growing soils in India have moderate to severe P deficiency. This is accentuated by the fact that a major part of rice growing soils like uplands and acid soils have high P-fixing capacity, resulting in reduced availability of P in soils (Vance et al., 2003). Even, in irrigated conditions, in the recent years, due to increasing cost of P fertilizers, the application of P fertilizers has reduced significantly, resulting in low to moderate deficiency of the nutrient. Fortunately, rice crop has significant genetic variability for low soil P tolerance (Ismail et al., 2007) and the genetics of low P tolerance is known to be controlled by a major QTL named Pup1 and several minor QTLs (Wissuwa et al., 1998). Pup1, originally identified from the Indian upland rice genotype, Kasalath is associated with P uptake efficiency and it was characterized to increase the root growth and biomass under low soil P conditions significantly (Heuer et al., 2009). The QTL has since then been fine-mapped and cloned (Gamuyao et al., 2012) and closely linked and functional markers are available for marker-assisted selection (Chin et al., 2011; Gamuyao et al., 2012). Utilizing these markers, we have successfully transferred Pup1 into three Indian mega-varieties of rice, which are highly sensitive to low soil P, *viz.,* Improved Samba Mahsuri, MTU1010 and IR64.

The improved breeding lines of these varieties were observed show better performance under low P soil with more number of productive tillers, better rooting and gave 30-50 % higher yield as compared to their original parents (*i.e.,* Improved Samba Mahsuri, MTU1010 and IR64). Significantly, under normal plots, the lines were observed to be similar to their recurrent parents for most of the agro-morphological traits (Anila et al., 2014). Two selected lines from each genetic background have been nominated for AICRIP trials recently. Through an extensive screening of more than 500 rice lines, we have identified and established that the rice varieties Rasi and Wazuhophek have high level of tolerance to low soil P and are devoid of Pup1 (*i.e.,* they possess novel mechanism of resistance). We have developed RIL mapping populations using Rasi and Wazuhophek as donors and Improved Samba Mahsuri as the recipient variety and screened for their tolerance to low soil P. Both the populations were observed to show normal distribution, with skewness towards tolerance indicating existence of a few major and several minor QTLs in the two donors.

Marker-assisted introgression of yield enhancing genes

Improvement in the yield potential of rice has proven to be the major strategy to increase global rice production particularly from irrigated rice to meet the demand of the growing population. An increase in yield potential of rice was achieved in the 1960s through the development of semi-dwarf varieties possessing the sd1 gene like IR 8, Jaya etc. However, it has been hard until today to break yield ceiling by using conventional breeding and selection strategies since the development of IR8 in tropical and sub-tropical environments (Peng et al., 1999). As a result, yield stagnation of newly developed rice varieties has been observed in several rice growing countries including India (Shobha Rani et al., 2013).

Five main research strategies are proposed to break the yield barrier in rice. These are: (1) genomic approaches to pyramid genes/QTLs for major yield component traits, (2) physiological approaches targeting source related traits, (3) marker-aided recurrent selection and genome wide selection, (4) utilization of natural variations for yield traits from wild *Oryza* species, and (5) increasing the heterosis level in hybrid rice production. However, it is prudent to use the genomics-assisted breeding strategy to pyramid genes controlling yield traits due to limited resources available to break the yield ceiling in rice. High yield potential contributed by several yield traits are controlled by complex genetic factors called quantitative trait loci (QTLs). Recently, several quantitative genes for yield were isolated by positional cloning from the fine-mapped regions of QTLs related to source and sink traits associated with increasing yield potential. The four main traits/genes, which can significantly enhance yield potential of Indica rice varieties are grain number per panicle (*Gn1a*) located on Chromosome 1 (Ashikari et al., 2005), panicle size and branching (*OsSPL14*) located on Chromosome 8 (Miura et al., 2010), strong culm (*SCM2*) located on Chromosome 6 (Ookawa et al., 2010) and grain size and grain weight (*GW5*) located on Chromosome 5 (Weng et al., 2008). These genes have been functionally characterized and their cellular localization has been elucidated. Most importantly molecular markers specific for each of these genes is available for use in breeding programmes.

Under the ICAR-IRRI collaborative work plan (2012-2017 and 2017-2020), we are developing introgression lines in the genetic background of the popular variety, Improved Samba Mahsuri, Swarna, MTU1010 and NDR359 possessing the favorable alleles of the major yield enhancing genes- *Gn1a, SCM2, OsSPL14* and *GW5* through marker-assisted breeding. Several near-isogenic lines (NILs) possessing one or two of the above mentioned traits in different genetic background have been evaluated in both normal nitrogen and high nitrogen containing soils and we have started pyramiding the different NILs with each other for combining multiple traits aimed at development of rice varieties and hybrids possessing durable resistance against major pests and diseases, possessing better grain quality, input use efficiency and high yield.

CONCLUSION

Marker-assisted breeding (MAB) is a power tool in the hands of rice breeders for improvement of multiple-traits. Our research team has demonstrated the capability of MAB in improvement of agronomically important traits in rice like biotic stress resistance, abiotic stress tolerance, heterosis breeding related traits, grain quality and yield and several improved versions (*i.e.,*

value added versions) of varieties and hybrids have been developed. One such products developed by us through MAB, *i.e.,* Improved Samba Mahsuri is presently being cultivated in more than 1,50,000 ha in bacterial blight endemic areas, clearly making a difference in the lives of the farmers. Many more such innovative products are in the pipeline of evaluation and release and it is our understanding that marker-assisted selection will soon be an integral component of rice breeding. Some of the high throughput tools like, high-throughput genotyping using SNPs, availability of trait specific SNP markers and development of regional genotyping hubs will certainly revolutionize rice breeding in the near future.

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