Exploration of Novel Genes and Alleles for Effective Biotic Stress Management in Rice

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Abstract

Rice blast, is one of the most widespread and destructive diseases of rice throughout the world. Management of this disease is becoming difficult due to high degree of variability in pathogenicity of *M. grisea* isolates prevalent in India. In addition, the resistance in in some resistant cultivars which have one major blast-resistance gene is quite short in the field. Hence, breedin g for more durably resistant cultivars has become a priority in rice improvement programmes throughout the world. Wild species of *Oryza* (store house of the genes) have rarely been used for the identification of blast resistance genes. Introgression of major genes coupled with strong QTLs will be the best strategy to make durable blast resistance. To identify the QTLs from wild *Oryza* species, we screened 326 stabilized introgression lines (ILs) of PR114 and Pusa44, which derived from the cross of various accessions of six different wild species *viz*. *O. nivara, O. glumaepatula, O. rufipogon, O. glaberrima, O. longistaminata and O. barthii.* After stringent blast disease screening at Directorate of Rice Research (DRR) for 3 seasons, and hot spot multi locations across India in All India Co-ordinated Rice Improvement Programme trials (AICRIP 2012-13) along with subsequent allelism test through linked and gene specific markers for 10 important blast resistance genes has led to the identification of nine extreme leaf and neck blast resistant ILs.

Keywords: Rice, Novel gene, allele, biotic stress management

1. Introduction

Rice blast, is one of the most widespread and destructive diseases of rice throughout the world. Management of this disease is becoming difficult due to high degree of variability in pathogenicity of *M. grisea* isolates prevalent in India. In addition, the resistance in in some resistant cultivars which have one major blast-resistance gene is quite short in the filed. Hence, breeding for more durably resistant cultivars has become a priority in rice improvement programmes throughout the world. To achieve broad-spectrum resistance, considering the constantly changing population of Magnaporthe, deployment resistance genes are the most viable option. Hence, identification of superior alleles of the targeted genes has become a necessity before any gene pyramiding program for durable and greater resistance (Ramkumar et al., 2010). Host resistance to this disease is well documented and more than 90 rice blast resistance genes were identified from various sources (Miah et al., 2013); among them, nineteen genes have been cloned and characterized. Among those characterized genes, *Pikh* is one of the major blast resistant genes of India, is being deployed in many breeding programs, since it showed high resistance to wide range of pathogen populations exist across different ecosystem of rice (Sharma et al., 2002; Ramkumar et al., 2011), which has been renamed as Pi54 (Sharma et al., 2010). *Pi54* was originally characterized in an indica rice variety – 'Tetep' at chromosome 11L. The gene has a single exon, encoding a protein possessing Nucleotide Binding Site – Leucine Rich Repeat (NBS-LRR) domain (Madhav et al., 2005). Over the past two decades, a shift from an over-reliance on major R-genes to selection for polygenic quantitative resistance has shown success in some breeding programs. In recent years, however, increased severity of blast was observed in Indonesia, Vietnam, and the Philippines. This suggests either an erosion of resistance due to pathogen evolution or a lessening of screening efforts in breeding programs, or both. There is an urgency to maintain the stability of blast resistance in these production systems, and to intensify efforts to understand the genetic mechanisms underpinning durable resistance.

Here, I wish to briefly assess the situation of applying quantitative and qualitative resistance in breeding, and then discuss our attempts to built the resistance through use of molecular tools.

2. Identification of Novel Genes

Wild species of *Oryza* (store house of the genes) have rarely

been used for the identification of blast resistance genes except for the two genes i.e., *Pi9* and *Pi40.* Stable QTLs are known to provide durable resistance towards blast, since they offer partial resistance. Introgression of major genes coupled with strong QTLs will be the best strategy to make durable blast resistance. To identify the QTLs from wild *Oryza* sp, we screened 326 stabilized introgression lines (ILs) of PR114 and Pusa44, which derived from the cross of various accessions of six different wild species *viz*. *O. nivara, O. glumaepatula, O. rufipogon, O. glaberrima, O. longistaminata and O. barthii.* After stringent blast disease screening at Directorate of Rice Research (DRR) for 3 seasons, and hot spot multi locations across India in All India Co-ordinated Rice Improvement Programme trails (AICRIP 2012−13) along with subsequent allelism test through linked and gene specific markers for 10 important blast resistance genes has led to the identification of nine extreme leaf and neck blast resistant ILs. Among the nine ILs, IL-31 derived from *O. glumaepatula* was selected for further characterization. Using uniformly spread 499 SSR markers across genome; we precisely identified ~ 4.61% of wild species genome introgression present on chromosomes 3 and 7. QTL analysis was done using two mapping populations developed using IL-31(as donor parent) and BPT 5204 and CO39 as recurrent parents. Phenotyping for leaf and neck blast was carried out at DRR and North East India respectively. This analysis led to the identification of a large-effect QTL on chromosome-3contributing for 53% phenotypic variance. This QTL located in a region of 3 Mb (6.8Mb to 9.7Mb). Another QTL has been identified on chromosome 7 contributing for 35% of phenotypic variance spanning a region of 8 Mb (4- 12 Mb). The efforts are underway to fine map these QTLs and simultaneous introgression in to the popular ruling varieties. This may be the first ever report of QTLs for neck blast resistance from the wild *Oryza* species.

3. Allele Mining for Identification of Novel Alleles

The untapped alleles of this gene, which might have left behind during the process of evolution and rice domestication, may provide better level of resistance to rice blast than the allele which has been characterized in the cultivated genotype. To achieve this, we selected three important blast resistance gene, Pi54, Pib and Pita for allele mining study to dissect the natural polymorphism in wide range of germplasm. Besides wild species of *Oryza*, landraces have also not been domesticated much, despite the fact that they are sources of diversity. The North Eastern (NE) part of India is not only blast endemic region but also rich in natural diversity for many crop species including rice. Hence, we hypothesized that the landraces collected from NE India and wild *Oryza* sp. may harbor the novel alleles. The presence or absence of these genes in these selected materials was confirmed with the genotyping with linked molecular markers, allelism test and the phenotypic data. Using the available sequence information of reference allele and complete genome sequence, various novel alleles were amplified from the selected ecotypes submitted in NCBI GenBank. The allele mining results revealed the presence of these genes in wide range of *Oryza* species, which indicates that, these genes might have originated long before in the evolution and the conservation of alleles in various *Oryza* species for the long period may indicate that these genes should be a functional and important resistant genes and provides resistance to a wide range of pathogen spectrum (Wang et al., 2008). Through allele mining of Pi54 identified a 144-bp insertion or deletion (InDel) polymorphism in the exonic region of the gene. A PCR-based co-dominant molecular marker targeting the InDel, named Pi54 MAS, was developed. Pi54 MAS was observed to perfectly co-segregate with blast resistance in a mapping population with no recombinants. Validation of this marker in 105 genotypes which are either susceptible or resistant to rice blast disease showed that the marker is polymorphic in most of the resistant–susceptible genotype combinations and is more accurate than the earlier reported markers for Pi54. Now this functional, co-dominant marker is suggested for routine deployment in MAS of Pi54 in breeding programs (Ram Kumar, 2012)

True allele mining was followed for *Pita* which studying the nucleotide diversity at coding and non coding regions, which are known to affect the plant phenotype eventually. *Pita* was analyzed by allele and promoter mining strategy and its different allelic variants were discovered from landraces and wild *Oryza* species. Polymorphisms at allelic sequences as well as transcription factor binding motif (TFBM) level were examined. At motif level,MYB1AT is present in *Pita*^{Tadukan} and other resistance alleles, but was absent in the susceptible allele. Core promoter was demarked with 449 bp, employing serial promoter deletion strategy. Promoter with 1592 bp upstream region could express the gfp two fold higher than the core promoter. The identified Pita resistance allele (Pita^{Konibora}) can be directly used in rice blast resistance breeding programs. Moreover, characterization of *Pita* core promoter led to deeper understanding of resistance gene's regulation and the identified core promoter can be utilized to express similar genes in rice (Ram Kumar, 2014)

To better understanding of resistance gene regulation at the transcription level, dissection of the promoter content and analysis of the architecture of TFBMs was done for 206 entire NBS-LRR genes of Arabidopsis and 120 genes of rice using three highly reliable motif prediction tools. A total of 36 and 30 novel, strong TFBMs were discovered from NBS-LRR genes of rice and A. thaliana, respectively. All the motifs identified in these sequences were analyzed for their positional conservation and the possible motif network associations were also identified. Further, the probability of the presence of motifs in these NBS-LRR genes were validated and statistically tested. Although Arabidopsis NBS-LRR sequences showed 76.3% similarity with rice sequences at motif level, the analysis revealed that rice sequences have many unique TFBMs and are more evolved in gene

expression mechanisms. This study also provided a list of novel candidate motifs for these genes, which will be a good resource for experimental validation. A novel strategy of prediction of gene expression based on motif arrangement was also demonstrated in this study. The findings of this study, such as the motifs' positional conservation, architecture, etc. offered new biological insights into the role of TFBMs in the regulation of resistance genes (Ramkumar, 2014).

Using, MAB (Marker assisted breeding), we introgressed three effective major blast resistance genes of India viz*., Pi1, Pi2* and *Pi54* into the Swarna sub1,submergence tolerant variety and Improved samba Mahsuri (which has 3 Bacterial Leaf Blight). Marker Assisted Backcross procedure coupled with phenotypic election was followed to develop these lines. In this process we have developed the lines carrying single and double and three genes in the combinations. Further, considering the importance of biotic and abiotic stresses, genetic enhancement of elite upland rice variety Varalu (WGL 14377) was chosen for improvement. In this variety, grain yield under drought and resistance to rice blast disease was improved by introgression of two major blast resistance genes i.e. Pi1 and Pi54 and a major QTL for grain yield under drought stress i.e. qtl12.1 through MAS.

Other than blast, rice yields are affected by many abiotic stresses like salinity, drought, cold and heavy metal; theses tresses trigger up and down-regulate several genes through various transcription factors (TFs). Transcription factor binding motifs (TFBMs), located in the upstream region of the genes, associate with TFs to regulate the gene expression. Many factors, including the activation of miRNAs, which are encoded by genes having independent transcription units, regulate the gene expression. TFBMs in the regulatory region of miRNA sequences influence the miRNA expression, which in turn influences the expression of other genes in the cell. However, the current level of information available on TFBMs of miRNA involved in abiotic stress related defense pathway(s) is limited and in-depth studies in this direction may lead to a better understanding of their role in expression and regulation of defence responses in plants. We studied various aspects related to genomic positions of premiRNA, predicted of TSS and TATA box positions and identified of known, unique motifs at regulatory regions of all the reported miRNAs of rice associated with different abiotic stresses. Sixteen motifs were identified, of which nine are known cis-regulatory elements associated with various stresses, two strong motifs, (CGCCGCCG, CGGCGGCG) and five unique motifs which might play a vital role in the regulation of abiotic stresses related miRNA genes. Common motifs shared by miRNAs that are involved in more than one abiotic stresses were also identified. The motifs identified in this work will be a resource for further functional validation (Rama, 2013).

So what it takes to achieve durable resistance? First, we need to know what genes or chromosomal regions have phenotypic effects in a range of germplasm with records of good blast resistance. This will tell us the consensus regions important for blast resistance. Combining QTL analysis of durable resistance with major genes responsible for quantitative resistance. Second, we need multiple screening sites to validate the effectiveness of the reconstituted resistance? Third, we need integration of mapping and gene datasets and collaboration among breeders, pathologists and geneticists across institutions. This would be a worthy objective for implementation by an international working group.

Among several biotic constraints that rice crop encounters, the insect pest, yellow stem borer (YSB) is the serious threat causers not only in India but also in all parts of rice growing regions across the globe since sufficient level of host resistance is not available. Management of this major problem of rice is being entirely dependent on the use of chemical pesticides. Despite the numerous efforts, success on host plant resistance against this deadly biotic stress agent has not been achieved so far. With tremendous advancement in the field of biotechnology and our understanding in pest biology, many newer approaches based on pathogen derived resistance (PDR) have become available for imparting resistance in plants. In such cases, RNAi mediated resistance by targeting the key genes of pests involved in host-pest interaction shown to have significant promise to address this problem. The success of RNAi technology primarily relies on identification of suitable candidate genes to utilize them as targets. Presently, scarcity of suitable candidate genes for these biotic stress is become a major limitation for applying RNAi technology. Deep transcriptome analysis of pest (YSB) at various stages of infection process have given clues on effectors which have definite role in defense responses and the mechanism of controlling plant disease susceptibility. Knowing such effectors have made way in imparting resistance which was achieved by knocking down the effectors which have negative role in defense. We have designed artificial miRNAs of key pest genes and transferred these in to rice for testing those genes. *In vitro* boassays with these genes already showed their effectiveness. The efforts are in way to judicious use of all biotechnology tools in manage the biotic abiotic stresses in the rice which eventually bring the sustainability.

4. Conclusion

Wild species of *Oryza* (store house of the genes) have rarely been used for the identification of blast resistance genes. Introgression of major genes coupled with strong QTLs will be the best strategy to make durable blast resistance. Using uniformly spread 499 SSR markers across genome; we precisely identified ~ 4.61% of wild species genome introgression present on chromosomes 3 and 7. QTL analysis was done using two mapping populations developed using IL-31(as donor parent) and BPT 5204 and CO39 as recurrent parents.

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