

Genome Analysis of Rice Genetic Resources for the Identification of Novel Genes and Alleles for Biotic Stress Resistance

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Rice (*Oryza sativa* L.) is one of the important crops which is the source of staple food for more than 3 billion people all over the world. Rice is subjected to various biotic and abiotic stresses during different stages of growth and development which directly affect its yield. Among the biotic stresses, various insect pests and diseases such as rice blast, bacterial leaf blight, brown leaf spot and sheath blight are severe constraints for yield of rice plant. Among the biotic stresses rice blast disease caused by *Magnaporthe oryzae* is the most dreaded disease which infects rice plant at all developmental stages affecting mainly leaves, collars, nodes, panicles, shoots and roots. Deployment of resistance genes for the management of rice blast disease is one of the best options available for resource poor farmers (1). The cultivation of different plant species by human beings has lead to the evolution of superior genotypes with improved agronomic traits over a long period. However, owing to their inferior agronomic traits, wild germplasm of most of the cultivated crop plants has been unattended and yet to be exploited for crop improvement. Wild germplasm of rice are 'treasure trove' as they harbour many useful traits, such as tolerance to biotic and abiotic stresses particularly disease resistance. Many important genes have been identified and cloned from wild species of rice. These include resistance genes for rice blast *Pi9(t)* and from *O. minuta*, *Pi-40(t)*, *Pirf2-1(t)*, *Pi54rh* and *Pid-A4* from *O. australiensis*, *O. rufipogon*, *O. rhizomatis* and *O. rufipogon* respectively. We cloned *Pi54* (2) blast resistance gene from rice line Tetep and its orthologues *Pi54rh* (3) and *Pi54of* (4) were later cloned from wild species *O. rhizomatis* and *O. officinalis* of rice, respectively. Once genes are cloned and functionally validated, their alleles and orthologues can be cloned and characterised from landraces and wild species of rice using allele mining approach. Allele mining is mainly used to find the novel variant of that gene and identification of signatures within the genes. Single nucleotide polymorphisms (SNPs) are hailed as

the unique identifiers of choice in plant genetic analysis because of their co-dominant inheritance, biallelic nature, chromosome specific location and abundance in the genome. In addition, they are highly amenable to automation and have the ability to reveal hidden polymorphisms giving higher resolution.

In rice, the greatest allelic diversity has been commonly observed in the accessions of *Oryza* spp. Using PCR-based approach, we have analyzed lines of *O. sativa* for mining alleles of three blast resistant genes (*Pita*, *Pi54*, and *Piz(t)*). Allelic variants of the broad spectrum blast resistance gene, *Piz(t)* have been analyzed from 49 rice lines (comprising of Indian local landraces and cultivated varieties) selected after phenotyping across three blast hot-spot regions of the India (5). Nucleotide variations in terms of SNPs (Single Nucleotide Polymorphism) and InDels were higher in the *Piz(t)* locus analyzed in this study. SNPs in the form of transitions were more frequent than the transversions in the *Piz(t)* orthologues. Based on nucleotide polymorphism, 46 haplotypes have been identified, with major haplotypes forming three main haplogroups. Allelic variants of the broad spectrum blast resistance gene, *Pi-ta* have been analyzed from 48 rice lines (local land races and cultivated varieties) selected after phenotyping these lines across three eco-geographical blast hot-spot regions (6). Besides, *Pi-ta* orthologue sequences of 220 rice accessions belonging to wild and cultivated species (*O. rufipogon*, *O. barthii*, *O. glaberrima*, *O. meridionalis*, *O. nivara*, *O. glumepatula* and *O. sativa*) were retrieved from the database were also included in the study for a better *evo-devo* perspective of the diversity present in the gene and the selection pressures acting on this locus. Nucleotide variations (SNPs and InDels) were higher in the intronic region compared to the coding region. Based on nucleotide polymorphism, 64 haplotypes have been identified, with major haplotypes forming eight main haplogroups. The variants for rice blast resistance gene *Pi54* from a panel of 92 rice genotypes were also prospected (7).

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The *Pi54* alleles of landraces harbour substantially higher polymorphism as compared to the *Pi54* alleles of cultivated species, because of heterogenous nature of land races. Among the *Pi54* alleles of *indica* and *japonica* species the diversity was low in the alleles of *japonica* species. In the haplotype network, the 50 identified haplotypes were clustered in five major haplogroups and the rest as minor haplogroups. Based on indels and SNPs allele based markers were designed for their use in rice improvement programme using marker assisted selection.

References

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