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Molecular Screening of Rice (*Oryza sativa* L.) Genotypes for Bacterial Leaf Blight Resistance Genes using Trait-based SNP Markers

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ABSTRACT: Bacterial leaf blight (BLB) disease is one of the major diseases of rice, which can cause detrimental effect to the yield. To overcome this issue development of resistant varieties by utilizing resistant gene pool is the most effective strategy for the plant breeders. The current study was conducted in order to evaluate the potential of 182 rice genotypes against Bacterial leaf blight (BLB) at genotypic level. The trait-based SNP (Single nucleotide polymorphic markers) were employed to detect the presence or absence of BLB resistance genes (*Xa4*, *Xa7*, *Xa13* and *Xa21*) in the genotypes. The genotypic results revealed that, 131 genotypes showed the presence of at least any one resistance genes studied. Interestingly, 48 genotypes did not show presence of any of the four resistance genes. The genes *Xa4*, *Xa7*, *Xa13* and *Xa21* were identified in 132, 2, 3 and 2 genotypes with frequency of 72.52%, 1.09%, 1.64% and 1.09% of genotypes, respectively. Notably, one genotype possessed two resistance genes (*Xa4* and *Xa7*) and two genotypes possessed three resistance genes (*Xa4*, *Xa13* and *Xa21*). Furthermore, cluster analysis grouped the 182 genotypes into two clusters, in which cluster I had genotypes not carrying any of the resistance genes and cluster II had genotypes carrying at least one or more than one resistance genes. The identified genotypes carrying multiple resistance genes could serve as a potential resource for BLB resistance targeted rice breeding programmes.

Keywords: Bacterial leaf blight, Gene, Resistance, Rice and SNP.

INTRODUCTION

Rice (*Oryza sativa* L.) holds prime importance as a staple food crop cultivated across diverse agro-climatic regions around the globe. Over 90% of the global rice production and consumption occurs in Southeast Asia. Rice is the highly cultivated crop in India and consumed by nearly entire population of the country. To meet the increasing needs of approximately 5.0 billion consumers globally, considering an annual average population growth rate of around 1.5%, the demand for rice is projected to surge by 40% by 2030 (Khush, 2005). This increase in demand can only met if there would be surge in production and productivity in rice. Nevertheless, cultivation of rice is majorly affected by both biotic and abiotic factors. Biotic stressors encompass fungi, bacteria, viruses, nematodes, insects, parasites, weeds, rodents and birds existing within the environment. Among the various bacterial diseases affecting the rice crop, bacterial leaf blight is a major disease caused by *Xanthomonas oryzae pv. oryzae* (Xoo). This disease could lead to yield loss of 20 to 50% and is a potential harm to rice production (Singh *et al*., 2011). In country like India, it is a major disease affecting rice during South-West monsoon season. Bacterial leaf blight, a vascular disease, symptoms characterized by leaf drying and yellowing that initiate from the tips and progress downwards. The kresek symptom is the aggravated form of disease that occurs at the seedling stage, results in a partial or total crop failure. Until now, there are a total of 46 genes offering resistance to various strains of the BLB pathogen are available to use (Yugander *et al*., 2018). Notably, a few of those R genes, such as *Xa3*, *Xa4*, *Xa5*, *Xa7*, *Xa13* and *Xa21* have been widely used by many researchers for identifying resistant genotypes and introgression into elite varieties. *Xa4* gene was identified by Petpisit *et al*. (1977) which provided durable resistance throughout all stages of plant growth in several commercial rice varieties. *Xa7* was identified by Sidhu *et al*. (1978). Yoshimura *et al*., (1995); Zhang *et al*. (1996) identified and tagged the *Xa13* gene. *Xa21* was identified in wild species, *Oryza longistaminata*, by Khush *et al*. (1990). Depending only on conventional breeding methods presents numerous limitations, making it highly challenging to choose rice lines with multiple resistance genes. Therefore, molecular breeding approaches is helpful to fasten the process in identifying resistant lines with multiple genes. The several systematic studies have been carried out to identify resistant sources for BLB disease in rice. Singh *et al*. (2012) screened land races for BLB resistance using *Xa4*, *Xa13* and *Xa21*, and observed only presence of *Xa4* in 69% of the land races. Panwar *et al*. (2018) conducted molecular screening to identify the presence of major BLB resistance genes *Xa4*, *Xa5* and *Xa21* in landraces and local cultivars of Gujarat.

Presently, DNA/molecular markers have laid the way to screen large number of accessions for identifying genotypes for the presence of several genes/QTLs governing various traits in rice. Till now majorly, SSR markers were mostly used by researchers to screen and identify resistant lines at molecular level. Notably, single nucleotide polymorphic (SNPs) markers are indeed widely used due to their high-throughput nature, low mutation rates, genome-wide coverage and high frequency (Arif *et al*., 2019). Trait based SNP markers helps in assessing the genetic diversity of rice genotypes and facilitating the identification of biotic and abiotic stress resistant/tolerant genotypes. Therefore, prioritizing the breeding of resistant rice varieties is considered the most economical method to overcome the primary hurdle in achieving peak yield. Consequently, the exploration of new resistant plant materials and their utilization of exceptional gene reservoirs are essential for advancing rice resistance against BLB. In this context, our research aimed to screen and identify the genotypes having BLB resistance genes linked to yield and use them in a stress breeding programs for developing improved rice varieties for sustainable food production.

MATERIAL AND METHODS

Experimental location and plant materials. The present experiment was carried out at ICAR-NRRI, Cuttack, India. A set of 182 advanced breeding lines consisting of both *indica* and *japonica* sub species were used to identify resistant genotypes for BLB disease.

DNA extraction and genotyping. The 1k-RiCA (Rice Custom Amplicon assay) was utilized for SNP genotyping for the 182 genotypes. The DNA extraction and genotyping procedure was carried out by company, Intertek India private limited. Four BLB resistance genes (Table 1) were used for molecular screening of 182 genotypes.

Table 1: List of trait-based SNP markers used for screening against BLB resistance in rice.

Gene	Chromosome number	Favourable allele	Unfavourable allele
Xa4	11	T	G
Xa7	6	G	A
Xa13	8	T	C
Xa21	11	C	T

Genotype scoring and data analysis. Genotypes were scored using a numerical method: assigning one for favorable alleles and zero for unfavorable ones. The binary data matrix generated by SNP markers was subjected to further analysis using Darwin software version 6.0. The dissimilarity matrix was used as an input for analysis of clusters. Phylogenetic tree was formed following un-weighted pair group method of arithmetic means (UPGMA) tool.

RESULTS AND DISCUSSION

Molecular screening of all the 182 genotypes for BLB resistance using trait-based SNP markers. The present experiment was aimed to identify BLB disease resistant genotypes from advanced breeding lines using trait-based SNP markers. The results showed presence of less to moderate level of diversity for BLB disease resistance genes at molecular level (Fig. 1). Out of 182 genotypes screened at molecular level, 131 genotypes showed the presence of at least one resistance gene among the four genes used. Notably, one genotype possessed two resistance genes and two genotypes possessed three resistance genes. Interestingly, 48 genotypes did not show presence of any of the four genes studied. We observed presence of *Xa4* in 132 genotypes corresponding to frequency of 72.52% of the total screened genotypes and which is in highest frequency compared to other genes. Ashiba *et al*. (2020) detected the *Xa4* gene in 46 rice genotypes out of 100 genotypes using SSR markers. Similarly, Acharya *et al*. (2018) also reported the presence of *Xa4* gene in 25 genotypes among the 60genotypes evaluated and they also observed that presence of *Xa4* gene in highest number of genotypes compared to *Xa5* and *Xa7* studied by them.

The genes *Xa7* and *Xa21*, each were found to be detected only in 2 genotypes, corresponding to frequency of 1.09% of the genotypes screened. This indicates that, *Xa7* and *Xa21* might be rare or specific to certain genotypes within the studied population. This rarity could depict the specialized functions or selective advantages for those two genotypes. Our results are on par with outcomes of Sabar *et al*. (2016), who also able to detect *Xa21*gene only in two germplasm lines out of 80 germplasm used. The gene *Xa13* was detected in only three genotypes, corresponding to frequency of 1.64% out of all the genotypes studied. Singh *et al*. (2012) also did not observe allelic status of *Xa13* gene in any of the land races screened for BLB resistance. Similarly, Baksh *et al*. (2018) also did not observe the presence of *Xa13* gene in rice cultivars studied. The above all supported studies used SSR markers for molecular screening. The presence or absence of specific genes among these genotypes could indicate natural genetic diversity within the population. Understanding the distribution and prevalence of specific genes within a population can provide valuable insights into genetic diversity, evolutionary patterns, and potentially useful traits for various purposes like breeding programs or understanding the mechanisms underlying the disease resistance.

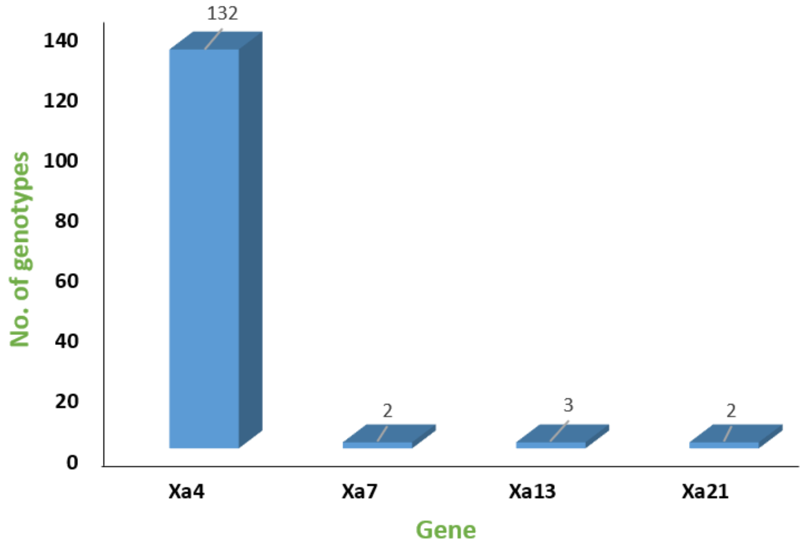

Fig. 1. Number of genotypes showing the presence of each BLB resistant gene.

Our results also revealed the genotypes containing multiple resistance genes in either combination of two or three genes. One genotype, RL-26possessed two BLB resistance genes (*Xa4* and *Xa7*) and two genotypes (RL-131 and RL-141) possessed combination of three resistance genes (*Xa4*, *Xa13* and *Xa21*). The presence of multiple genes indicated the suitability as donor in marker assisted improvement for BLB resistance. Acharya *et al*. (2018) detected five genotypes showing presence of both *Xa4* and *Xa7* genes while screening for BLB resistance genes in rice genotypes. Biswas *et al*. (2021) observed combination of *Xa4*, *Xa13* and *Xa21* genes in 16 recombinant introgressed lines developed by crossing Ciherang with IRBB60.

Cluster analysis. Cluster analysis was used to uncover the genetic connections and similarities among the various rice genotypes based on the four BLB resistance genes. UPGMA dendrogram separated 182 advanced breeding lines into two major clusters (Fig. 2). The 48 genotypes were grouped in Cluster I and those genotypes did not possess any of the BLB resistance genes. It indicated that, the resistant genotypes are present in different cluster group having high dissimilarity than other genotypes and clearly notifies that resistant genotype had high diversity with BLB resistance. The Cluster II consisted of remaining 134 genotypes and those possessed one or more than one BLB resistance genes.

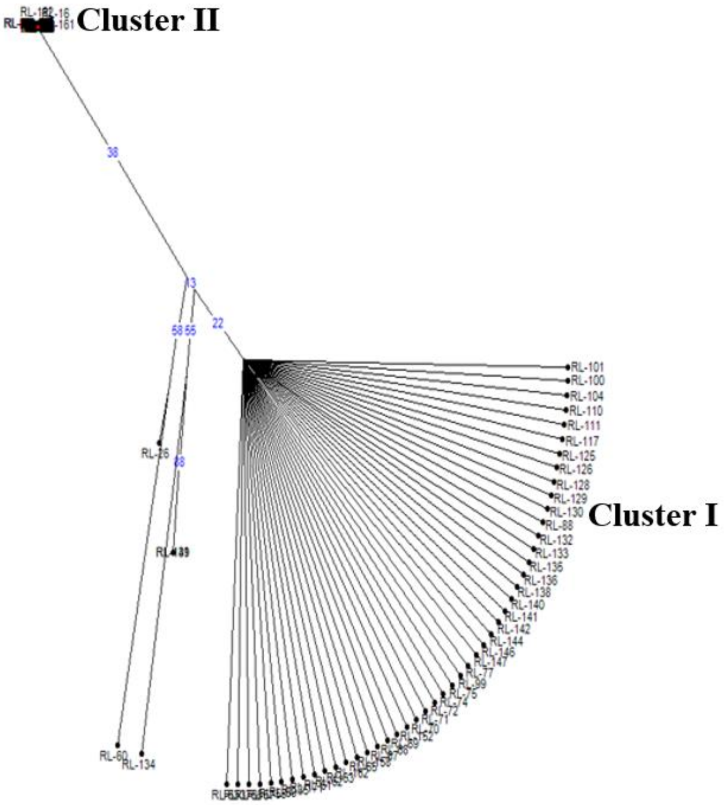

Fig. 2. UPGMA dendrogram showing clustering pattern of 182 rice genotypes based on four BLB resistance genes.

CONCLUSIONS

The present study highlighted that molecular validation is the fast and essential strategy to explore the variation of various rice genotypes for their potential against BLB disease. The genotypes having any one of the resistance gene can be utilized in crop improvement activities targeting for BLB resistance. The two identified multi-genic lines, which contain the genes *Xa4*, *Xa13* and *Xa21* would be a potential donor parents for developing BLB resistant varieties through marker assisted improvement programme in rice. Having multiple genes conferring resistance is a robust approach in the face of climate change, ensuring sustainable food security. This study further suggests that genotypes indicating resistance at molecular level should be evaluated at field level for further confirmation.

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Conflict of Interest. None.

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