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Characterization of Traditional Rice Varieties for Leaf Blast Resistant Genes *Pi5*, *Pi54*, *Pi9* and *Pi2* using Gene Specific Markers

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Abstract

Magnaporthe oryzae poses a serious risk to rice growing regions worldwide. To combat this, future breeding efforts that aim to develop resistant varieties will need to identify and screen blast-resistant cultivars from existing germplasms. Thus, present study aimed to identify four major blast resistant genes (*Pi54*, *Pi5*, *Pi2* and *Pi9*) in 20 traditional rice varieties using functional and linked markers. Results of the present study identified that fifteen traditional rice landraces were found to possess at least one resistant gene and three traditional landraces (*Aanaikomban*, *Chenellu* and *Jai Sri Ram*) had two resistant genes. These identified traditional rice landraces could be used as promising donor against rice blast disease for future rice breeding programmes to develop superior cultivars.

Keywords: Gene linked markers, Functional marker, Blast, Traditional varieties

Introduction

Rice blast disease is caused by the fungus *Magnaporthe oryzae*, which remains one of the important biotic stress on rice production worldwide, with yield losses of up to 37.8% (Khamari, 2020). Blast disease is an ascomycete fungus having novel G-protein coupled receptors called CFEM for its pathogenicity (Dean *et al.*, 2005). It is found in approximately 85 countries across all continents, in both lowland and upland conditions (Hasan *et al.*, 2015) and considered as most destructive pathogen of rice worldwide (Dean *et al*., 2005).

Although chemical fungicides are expensive, they have proven to be helpful in the management of disease (Sahu *et al.*, 2018). Developing a blast resistant variety would be the most ideal and cost effective approach. For blast resistance, nearly 102 genes (27 cloned) and 347 QTLs discovered so far (Khan *et al.*, 2018). However, some of the identified genes had a broad spectrum resistance against blast. *Pi54*, formerly referred to as *Pikh* provides resistance against several blast disease strains, as reported by Sharma *et al*. (2005). In 2008, Xu *et al.* discovered the *Pi54* gene in the "Tetep," and mapped on chromosome 11. Further, it has been cloned using a map-based technique (Ramkumar *et al.*, 2011). Another major resistance gene, *Pi9* that offers broad-spectrum protection against several blast fungus strains (Qu *et al.*, 2006). A comparative analysis revealed that the gene *Pi9* was shown 52% resistance while *Pi54* gene showed 43% resistance (Jain *et al.*, 2019). It has been documented that the *Pi2/9* locus in both wild and cultivated rice species contains at least eight R genes (Jiang *et al.*, 2012). *Pi5* has been shown to impart resistance to a huge number of *M. oryzae* strains that were obtained from the Philippines and Korea (Han, 2001).

A large number of germplasms may be quickly and simply evaluated for the existence of blast resistance with the help of gene-specific molecular markers without the need for a comprehensive disease screening (Fjellstrom *et al.*, 2004). Traditional landraces which act as a reservoir,

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having a superior alleles for various stresses (Shanmugam *et al.*, 2023). These have not been widely utilized or incorporated into modern varieties. Molecular screening using gene specific markers could be helpful in identifying desirable genetic resources to be utilized in rice breeding programmes in order to develop superior cultivars (Thete *et al*., 2023). Numerous PCR-based markers have previously been developed and are being utilised to mine different germplasm panels that hold untapped resources of distinct alleles for blast resistance genes (Yadav *et al.*, 2019). Various researchers have been screened large germplasm sets using blast gene specific markers (Sooklim *et al.*, 2022; Jeevan *et al.*, 2023; Panja *et al.*, 2023). Thus the present study aimed to identify the landraces possessing targeted blast resistant genes (*Pi5, Pi54, Pi9* and *Pi2*) using gene specific markers. The current study's findings will aid in the discovery of new, important donors for blast resistance, enabling the creation of long-lasting blast resistant cultivars in India.

Materials and Methods

Plant Materials

A panel of 24 traditional landraces were utilized (Table 1). Seeds of the selected panel were obtained from the Tamil Nadu Rice Research Institute (TRRI), Aduthurai, Thanjavur, Tamil Nadu. Rice seeds were grown in a glasshouse facility at TRRI, Aduthurai for DNA extraction.

DNA Extraction

Using the CTAB approach, the genomic DNA was isolated from fresh young leaves (Doyle and Doyle, 1990). The quantity of isolated DNA was quantified using a Nanodrop ND-1000 spectrophotometer. DNA was diluted with sterile water to make the final concentration of DNA of about 50 ηg μl^{-1} for PCR assay after quantification.

Table 1: List of traditional varieties used in the study

Sl. No.	Traditional varieties	Sl. No.	Traditional varieties
1.	*Aanaikomban*	11.	*Chithirai Kar*
2.	*Aarupathamkuruvai*	12.	*Chittimutyalu*
3.	*Aathurkichadi samba*	13.	*Edakkal*
4.	*Adukkan*	14.	*Gandakasala*
5.	*Athurkichadi*	15.	*GEB-24*
6.	*Chandaikar*	16.	*Gedumani*
7.	*Chenellu*	17.	*Illupai poo Samba*
8.	*Chinkinikar*	18.	*Iravai Pandi*
9.	*Chinna Punchai*	19.	*Jai Sri Ram*
10.	*Chinnar*	20.	*Kuliadichan*

PCR Amplification

PCR amplification using gene linked primers (Table 2) of *Pi54* (Ramkumar *et al.*, 2011), *Pi9* (Qu *et al.*, 2006), *Pi5* (Lee *et al.*, 2009) and *Pi2* (Tian *et al.*, 2016) were done. Using a 10 µl PCR reaction mixture that contained 1 µl (50 ηg μl^{-1}) of genomic DNA, 1 µl of primer (2 mM), and 8 µl of commercial PCR master mix (1X), PCR amplification was performed in a Veriti master cycler. The thermal cycler profile included five minutes of initial denaturation at 94 °C, thirty seconds of denaturation at 94 °C, annealing at 55 °C, one minute of extension at 72 °C, and 10 minutes of final extension at 72 °C. 2.5% agarose gel in 0.5 × TBE (Tris-borate-EDTA) buffer was used to separate the PCR products, and the size of an amplified fragment was calculated.

Table 2: List of markers used in the study

Sl. No.	Gene	Chr. No.	Marker	Primer sequence (5'- 3')
1.	*Pi54*	11	Pi54MAS	F: CAATCTCCAAAGTTT-TCAGG
				R: GCTTCAATCACTGC-TAGACC
2.	*Pi5*	9	JJ803	F: AAGTGAGCAT-CCAGTGCCTAATGA
				R: AGCCGGTGCT-CATAACACGTATTA
3.	*Pi9*	6	NBS4	F: ACTTTGTTGTGCTT-GATAAC
				R: ATGGTGAACGGTA-TCTGTAT
4.	*Pi2*	6	9-PRO-F	F: TGATTATGTTTTTT-ATGTGGGG
				R: ATTAGTGAGATCCATT-GTTCC

Results and Discussion

Large-scale genetically homogeneous variety cultivation put pathogen populations under intense selection pressure which increased the susceptibility of the cultivars to majority of the biotic stresses. Rice production has always been threatened by changing climate and the emergence of new, virulent races (Yadav *et al.*, 2019). In this study, we investigated twenty rice landraces for their allelic pattern of targeted blast resistant genes.

Genotypic screening with the Pi54 MAS (gene linked marker) for the *Pi54* resistant gene were found that only four traditional rice varieties, *viz*., *Aanaikomban*, *Chandaikar*, *Chinnar* and *Iravaipandi* had targeted resistant allele with 216 bp allele size (Figure 1). A 14 traditional landraces were shown susceptible allele with allele size of 359 bp, while *Chenellu* and *Chinnapunchai* had a both resistant and susceptible allele (Figure 2).

Amplification of *Pi5* gene using JJ803 marker for 20 traditional rice varieties identified four genotypes (*Athurkichadi*, *Chittimutiyalu*, GEB-24 and *Illupai poo samba*) with *Pi5* resistant allele (300 bp). Previous research found that *Pi5* gene in 4 Manipur landraces (Mahender *et al.*, 2012), 60 Karnataka landraces (Ingole *et al.*, 2014) and 83 landraces (Yadav *et al.*, 2019). The genes *Pi3* and *Pii* are allelic to *Pi5*

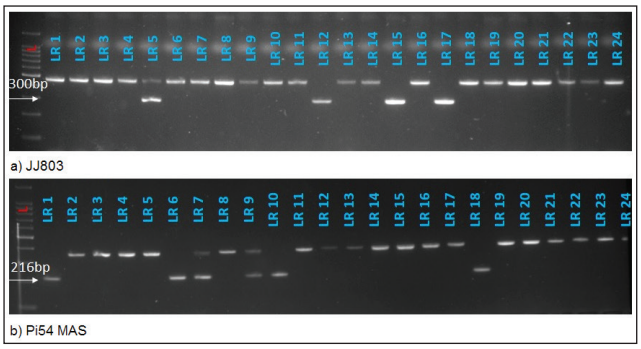

Figure 1: Amplification pattern of JJ 803 and *Pi54* MAS markers

Sl. No.	Genotypes	JJ 803	Pi54 MAS	9-Pro-F	NBS 4
1	*Aanaikomban*				
2	*Aarupathamkuruvai*				
3	*Aathurkichadi samba*				
4	*Adukkan*				
5	*Athurkichadi*				
6	*Chandaikar*				
7	*Chenellu*				
8	*Chinkinikar*				
9	*Chinna Punchai*				
10	*Chinnar*				
11	*Chithirai Kar*				
12	*Chittimutyalu*				
13	*Edakkal*				
14	*Gandakasala*				
15	*GEB-24*				
16	*Gedumani*				
17	*Illupai poo Samba*				
18	*Iravai Pandi*				
19	*Jai Sri Ram*				
20	*Kuliadichan*				

Figure 2: Allelic status of blast resistance genes [Green colour represents resistant allele, Red colour represents susceptible allele and Yellow colour represents heterozygotes]

(Jeon *et al.*, 2003; Yi *et al.*, 2004). Lee *et al.* (2009) identified that JJ803 marker was co-segregated with *Pi5* mediated resistance at 0 cM which was derived from JJ80-T3 dominant marker.

A total of eight out of 20 traditional landraces were reported to have *Pi9* resistant allele. *Pi9* gene has been found to co-segregate with functional marker NBS 4 (Qu *et al.*, 2006). Allele of *Pi9* (1Kb) was found in *Aanaikomban*, *Aarupathamkuruvai*, *Adukkan*, *Chenellu*, *Edakkal*, *GEB-24*, *Jai Sri Ram* and *Kuliadichan*. Allele specific InDel marker 9-Pro-F was used to genotype for the blast resistant gene *Pi2* (Tian *et al.*, 2016). Only one traditional variety *Jai Sri Ram* had a *Pi2* resistant allele while all other traditional varieties possess non-*Pi2* allele.

Of the selected traditional varieties, *Aanaikomban* and *Chenellu* had a *Pi54* and *Pi9* genes while *Jai Sri Ram* possessed *Pi2* and *Pi9* resistant alleles. Utilizing these varieties in future breeding programmes to introgress the multiple resistant genes would be ideal to develop blast resistant varieties.

Conclusion

Present study revealed that screening traditional landraces which are considered as "treasure of breeders" is crucial as it harbours wide range of genetic variation and possesses superior alleles for major biotic stresses. Molecular characterization using gene linked and functional markers helped us to identify the traditional varieties such as *Aanaikomban*, *Chenellu*, *Jai Sri Ram* which harbours two genes for blast resistance. The identified resistant varieties are need to be evaluated further under natural (hotspot) locations as well as artificial (blast nursery) conditions. The data produced here has increased our understanding of the importance of gene linked markers, which has great potential for marker-assisted breeding to increase rice blast resistance.

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