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SCREENING OF NELLORE MAHSURI RICE VARIETY FOR BACTERIAL LEAF BLIGHT TOLERANCE USING GENE SPECIFIC SSR MARKERS

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ABSTRACT

Nellore Mahsuri (NLR34449) is a medium fine grain rice variety and mostly adopted by many of the farmers. However, the variety is resistant to blast but susceptible to bacterial leaf blight (BLB). Phenotype screening had been done for two varieties to determine BLB score of Nellore Mahsuri (NLR34449) in comparison to a known check RPBio226 (improved samba Mahsuri), which is having three broad spectrum tolerant BLB genes viz., *Xa21, xa13* and *xa5.* Genotyping of two varieties was studied using three SSR gene specific markers *viz*., pta248, *xa13* pro, *xa5*FS, linked to the BLB resistance genes viz. *Xa21, xa13* and *xa5, respectively*. Allelic scoring for these markers revealed the abscence of these three broad spectrum genes in NLR34449. Hence, introgression of these genes would improve the tolerance levels of this popular variety.

KEYWORDS: Bacterial leaf blight (BLB), tolerance, SSR markers

INTRODUCTION

Rice *(Oryza sativa L.)* is an important food crop that serves as a major carbohydrate source for nearly half of the world's population. India is the second largest producer of rice after China with an area of over 43.86 m ha and production of 104.80 mt (Directorate of Economics & Statistics, 2015). Bacterial leaf blight causes significant yield losses in severely infected fields ranging from 20 to 30 per cent, but this can reach as high as 80 to 100 per cent (Baliyan *et al*., 2016) and also severely affects the grain quality. Nellore mahsuri is a fine grain rice variety, high yielding, short duration, non-lodging resistant to blast but it is susceptible to BLB. NLR34449 from Agriculture Research Station, Nellore, ANGR Agricultural University.

Chemical control for the management of BLB is not effective. Therefore, host plant resistance offers the most effective, economical and environmentally safe option for management of BLB (Sombunjitt *et al.,* 2017). Therefore, most researchers are interested in identifying a resistant cultivar and searching for available resistance genes against BLB disease Currently, 40 genes which confer resistance to various *Xoo* strains have been designated in a series from *Xa1* to *Xa40* (Sombunjitt *et al*., 2017 and Kim *et al*., 2015). A rice cultivar carrying many resistance genes can show a broad spectrum and higher level of resistance to pathogens than a cultivar with a single resistance gene (Rajpurohit *et al*., 2010).

MATERIALS AND METHODS

Two rice varieties *i.e.,* NLR34449 (Nellore Mahsuri) and RPBio226 (Improved Samba Mahsuri) were used as parents in this work. Characters of two varieties listed in the Table 1.

Table 1. Morphological characters of NLR34449 and RPBio226

Parent	Special features	Yield (qha^{-1})	Duration
NLR 34449	A Popular, high yielding , Fine grain and short duration rice variety, but susceptible to BLB	50.4qha^{-1}	120-125 days
RPBio 226	Resistant to BLB	46.3qha^{-1}	130-135 days

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Phenotypic Screening for bacterial leaf blight resistance

During *kharif* 2017, parents NLR34449 and RPBio226 were screened against bacterial leaf blight pathogen. The virulant isolate of *Xanthomonas oryzae pv. oryzae (Xoo)* was collected from Agricultural Research Station (ARS), Nellore was used for phenotypical screening of two varieties under glass house conditions. The rice plants were clip-inoculated with a bacterial suspension of 10^9cfu/mL at the maximum tillering stage (50 d after transplanting).Approximately 10 leaves per plant were inoculated, In NLR34449 BLB symptoms were observed 2nd day onwards from the day of inoculation. RPBio226 hasn't shown any symptoms. Nellore Mahsuri was found susceptible for bacterial leaf blight, while improved samba mahsuri was resistant, since it carries three resistance genes xa5, xa13 and Xa21 for bacterial leaf blight resistance. Disease severity is assessed based on lesion length measurement or estimation of diseased leaf area. Disease score was recorded based on lesion length as per 1-9 scale of standard standard evaluation system (SES) score (IRRI, 2013) as shown in Table 2.

Fig 1.Bacterial leaf blight disease screening of RP Bio-226 and NLR34449 through cut inoculation method.

Table 2. Scale for rating BLB resistance as per the standard evaluation system.

Scale	Diseased Leaf Area (%)	Description
1	1 - 5	Resistant (R)
3	6 – 12	Moderately Resistant (MR)
5	13 – 25	Moderately Susceptible (MS)
7	26 – 50	Susceptible (S)
9	> 50	Highly Susceptible (HS)

Genotyping

Genomic DNA was extracted from freshly collected leaves of the two parents, NLR34449 and RPBio226 by using CTAB method. The PCR amplification using SSR primers was carried out using sterilized thin walled PCR tubes (0.2 ml). PCR protocol for amplification of markers (pTA248, *xa13* pro, *xa5*FS) as described by Sundaram *et al.* (2008) was adopted. As regards to the newly designed functional markers for xa13 and xa5, PCR was performed using 0.1 µl of(5U/µl) Taq DNA polymerase (abm),2 µL of (100ng/ µL) DNA template DNA, 0.5 µL OF 5mM of each primer, µl of 1Mm dNTPs, and 1 µl 10X PCR buffer (abm) in 25 µL reaction volume with a thermal profile of 94 °C for 5 min (initial denaturation), followed by 30 cycles of denaturation at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, extension at 72 °C for 1 min and a final extension of 7 min at 72 °C. The amplified products were electrophoretically resolved on a 2.0 per cent agarose gel, stained with ethidium bromide and visualized in a gel documentation system (Alpha Innotech, USA). However, with respect to the multiplex PCR assay for simultaneous detection of Xa21, xa13 and xa5, the protocol was essentially same as described above, except that annealing was done at 57 °C and the amplified products were resolved in 2 per cent agarose gels. The primer sequences for the functional markers for the three resistance genes are given in Table 3

Table 3. Gene specific primers and their markers

Gene	Primer name	Primer Sequence	AT
xa13	xa13-prom F	GGCCATGGCTCAGTGTTTAT	57°C
	xa13-prom R	GAGCTCCAGCTCTCCAAATG	
Xa21	Pta248 F	AGACGCGGAAGGGTGGTTCCCGGA	57°C
	Pta248 R	AGACGCGGTAATCGAAAGATGAAA	
xa5FM	xa5-SF	GTCTGGAATTTGCTCGCGTTCG	57°C
	xa5-SR	TGGTAAAGTAGATACCTTATCAAACTGGA	
	xa5-RF	AGCTCGCCATTCAAGTTCTTGAG	
	Xa5-RR	TGACTTGGTTCTCCAAGGCTT	

RESULTS AND DISCUSSION

The DNAs from the recurrent parent NLR34449 and the donor parent RPBio226 (for *xa21*, *xa13* and *xa5*) were used to determine marker polymorphism. The reported sequenced tagged sites (STS) markers xa5FS, xa13pro and Pta248 showed polymorphism between recurrent parent Nellore Mahsuri and donor parent RPBio226. The primer pair pta248 amplified fragments of 950bp in the resistant parent RPBio226 (improved samba mahsuri), while that from the susceptible parent NLR344449 was 660bp. With respect to the primer pair, xa13-prom, RPBio226 (improved samba mahsuri), amplified a 500bp fragment, while NLR34449 amplified a 250bp. Similarly, with respect to the marker xa5FS, a fragment of 313bp was amplified in NLR34449, while a 420bp and 150bp fragments were amplified in RPBio226. Parental polymorphism between NLR34449 and RPBio226 (improved samba mashuri) was observed with the *xa4, xa5, xa8, xa13, xa21, xa23* gene specific markers (Fig. 2) of which, all the genes, except *xa8,* showed polymorphism between the parents.

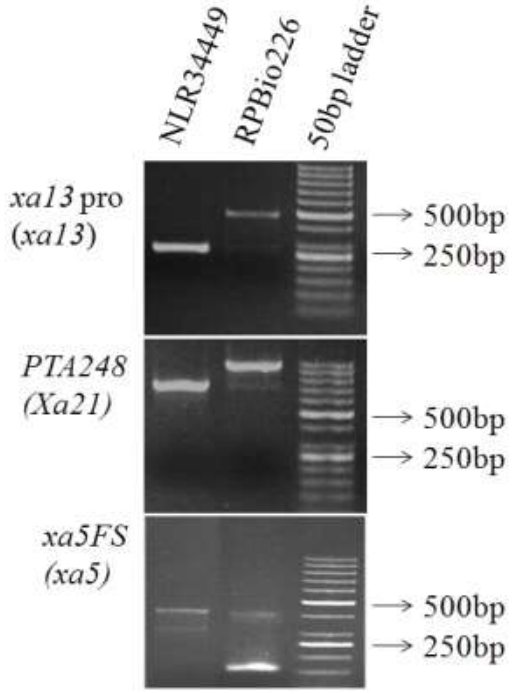

Fig 2. Parental polymorphism for xa5, xa13 and Xa21 gene using linked marker xa5FS, xa13pro and Pta248, respectively.

CONCLUSION

Based on allelic pattern of BLB resistant genes NLR34449 (Nellore mahsuri) rice variety is susceptible to BLB. Major BlB genes are transferred into NLR34449 through Marker assisted introgression and developed into a resistant variety.

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