



Molecular Screening and Agronomic Trait Characterization of NLR 34449 X ISM Derived Population for their resistance against Bacterial Blight Disease

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Abstract

Bacterial Blight (BB) disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is a major limiting factor amongst the diseases of rice in India. NLR34449 is a very popular high yielding variety. It is a popular variety not only in Andhra Pradesh but also in the neighbouring states of South India, but highly susceptible to the bacterial blight disease. A total of 802 BC₁F₂ plants derived from the cross NLR 34449 and ISM were phenotypically screened for bacterial blight resistance, 687 were found to be resistant to BB. Genotyping of these plants revealed ten homozygous positive plants for all the three target BB genes viz., *Xa21*, *xa13* and *xa5*. They were further assessed for key agro-morphological traits. viz., Days to 50% flowering, plant height, number of panicles per plant, thousand grain weight and grain yield per plant. Almost all the traits exhibited variation for key agronomic traits and among them, five plants were found to be performing well not only for bacterial blight resistance but also found to be superior in terms of key agronomic traits like thousand grain weight and single plant yield and flowered earlier than the parents as well. These identified plants will be advanced for further evaluation.

Keywords: Bacterial blight (BB), *xa21*, *xa13*, *xa5*, NLR34449, ISM

Introduction

Rice (*Oryza sativa* L.) is the primary staple food in many countries and is one of the most important cereal crops grown all over the world. In India, 122.27 million tonnes of rice is being produced in 45.07 million hectares with a productivity of 2713 kg ha⁻¹ (<https://desagri.gov.in>). In Andhra Pradesh, 7.89 million tonnes of rice is being produced in 2.32 million hectares, with a productivity of 3395 kg ha⁻¹ (<https://desagri.gov.in>). Current global yield increase rates (1.0% per year) of rice are insufficient to meet food demand for the estimated nine billion people in 2050 (Khush, 2001).

Major production constraints of rice in the country are biotic stresses like BB, Blast and abiotic stresses like drought, salinity, and low soil phosphorous coupled with new emerging challenges from climate change, increasing cost of cultivation, and socioeconomic changes. The only way to sustain rice production to meet the increasing population demand is host plant resistance with increased productivity under disease endemic areas. Among the biotic stresses, Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* is most destructive and drastically reduces the crop yield by up to 50%

(Khush *et al.*, 1989) or even up to 80% in some areas of Asia (Singh *et al.*, 2001).

Improved cultivars carrying resistance genes have been the most effective and economical strategy to control BB disease (Suh *et al.*, 2013). Moreover, deployment of rice cultivars that have multiple BB resistance genes is expected to enhance the durable resistance against BB. To date, 42 BB resistance genes have been identified from cultivated, mutant population and wild rice species (*O. longistaminata*, *O. rufipogon*, *O. officinalis* and *O. Minuta*) designated from *Xa1* to *Xa42* conferring resistance against various strains of *Xoo* (Brar and Khush, 1997; Lee *et al.*, 2003).

Keeping in view, the importance for development of resistant cultivars, the present investigation was carried out to identify BB resistant cultivars along with better yield and yield attributes in BC₁F₂ generation derived by crossing a popular dwarf, high grain number Nellore Mahsuri (NLR34449) with Improved Samba Mahsuri (ISM) BB resistant (harbouring *Xa21*, *xa13* and *xa5*), high yielding, fine-grain type with good cooking qualities.

Materials and Methods

Plant material

The plant material for the present investigation includes two high yielding fine grain type varieties *viz.*, BB resistant Improved Samba Mashuri (ISM) and BB susceptible Nellore Mahsuri (NLR34449) which were used as parents to develop BC₁F₂ population. Varietal characteristics of parents were presented in **Table 1**. These were evaluated for phenotypic screening against bacterial blight (BB) and for key agronomic traits. The donor parent ISM, recipient parent NLR 34449, resistant and susceptible checks were compared along with BC₁F₂ plants.

Table1: Salient Features of parents used in developing BC₁F₂ population

S. No.	Characteristics	Recurrent parent (Nellore Mahsuri)	Donor parent (Improved Samba Mahsuri)
1.	Average days to heading	95-100	95-110
2.	Plant type	Dwarf	Semi dwarf
3.	Average days to maturity	120-125	135-140
4.	Plant height (cm)	75-80	86-95
5.	Panicle exertion	Exerted	Partial exertion
6.	Grain type	Medium slender	Medium slender

Methodology

During *rabi* 2017, Improved Samba Mashuri (ISM) was crossed with Nellore Mahsuri (NLR34449) (**Figure 1**) to develop F₁ seed. True F₁ plants were identified with the help of marker assisted selection using gene specific markers *viz.*, pTA248 (for *Xa21*), *xa13* prom (for *xa13*) and *xa5FM* (*xa5*). These identified true F₁s were then backcrossed with NLR34449, the recurrent parent. Positive BC₁F₁s heterozygous for all the three targeted genes were selfed to produce BC₁F₂, which were further confirmed genotypically and homozygous positive plants were identified and evaluated for key agronomic traits.

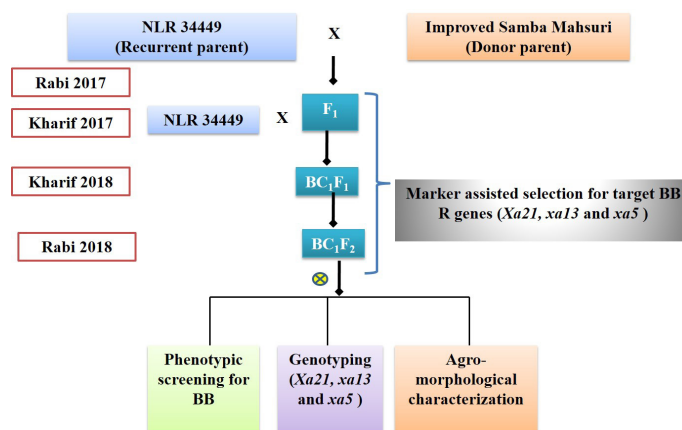


Figure 1: Schematic representation of work strategy deployed in the present study

DNA Extraction and PCR Amplification

DNA was extracted using two weeks old fresh leaf samples collected from nursery with modified CTAB



extraction method (Doyle and Doyle, 1987) with few modifications. PCR was carried out for three markers with 10 µl reaction volumes, with 5 picomoles of each forward and reverse primers, 1X PCR master mix (Thermo scientific) and 30-50 ng of each genomic DNA sample. PCR conditions for pTA248, *xa13prom* and *xa5FM* were - initial denaturation at 94 °C for 5 minutes, followed by 35 cycles of denaturation 94 °C for 30 seconds, 55 °C for 30 seconds, annealing and extension 72 °C for 1 minute and final extension was at 72 °C for 7 minutes. Marker polymorphism was visualized on 1.5% agarose gel electrophoresis.

Phenotypic screening against BB

Homozygous positive BC₁F₂ plants selected based on the marker assisted selection were planted in the experimental farm of ICAR-IIRR along with the parents (NLR34449 and ISM) at a spacing of 15 x 20 cm following the agronomic practices. At maximum tillering stage, *Xoo* culture (DXO-20) was adjusted at a concentration of 10⁸ cfu/ml (Preece, 1982) and inoculated tips of 3-4 leaves by clipping method following Kauffman *et al.*, (1973). After 15 days of inoculation scores were recorded based on the standard evaluation scale (IRRI, SES 2013).

Agronomic trait evaluation

Selected BC₁F₂ plants which were homozygous positive for all the target genes and with excellent resistance to BB were further evaluated for key agronomic traits along with recurrent (NLR34449) and donor (ISM) parents. Data was recorded for the key traits *viz.*, Days to 50% flowering, plant height, number of panicles per plant, grain yield per plant and 1000 grain weight and superior BC₁F₂s identified were further advanced.

Results and Discussion

BB is a serious disease of rice in India, particularly affecting the crop in the irrigated agro-ecosystem caused by the plant pathogenic bacteria *Xanthomonas*

oryzae pv. *oryzae* and is one of the devastating diseases of rice causing yield losses ranging from 74-81% (Sundaram *et al.*, 2009, Srinivasan and Gnanamanickam, 2005). When plants are infected at booting stage it results in poor quality grains with a high proportion of broken kernels. Breeding and development of resistant varieties carrying major resistance alleles have been the most effective and economical strategy to control BB with a minimal adverse effect on the environment (Huang *et al.*, 1997; Jena and Mackill, 2008; Singh *et al.*, 2001).

Host plant resistance has been considered as the most economical and eco-friendly strategy for management of biotic stresses (Hulbert *et al.*, 2001). For BB there is no effective chemical control method practiced, hence, the only durable strategy is to grow resistant varieties (Huang *et al.*, 1997). In the present investigation, a total of 802 BC₁F₂ plants derived from NLR34449 X ISM carrying the resistance dominant allele *Xa21* and recessive *xa13*, *xa5* genes were screened phenotypically and among which 687 were resistant, which were further screened genotypically with the help of targeted gene linked markers. Foreground selection of these plants resulted in the identification of 42, 39 and 10 positives for *Xa21* and *xa13* combination, *Xa21* and *xa5* combination and *Xa21*, *xa13* and *xa5* combination, respectively.

Previous reports of Shanti *et al.*, (2001) identified the triple homozygotes with the help of RG 136 and pTA 248, tightly linked to *xa13* and *Xa21*, respectively and SSR marker RM 122 linked to *xa5* markers. Same set of markers were used for *Xa21* and *xa13* genes whereas RG136 marker was used for *xa5* in reports of Sundaram *et al.*, (2008 & 2009). However, in our present study, we have used pTA248, *xa13prom* and *xa5FM* for foreground selection of *Xa21*, *xa13* and *xa5*, genes respectively. Similar observations were made by Rekha *et al.*, (2018).

All the three target genes that have been gone through in the present study have been cloned and characterized.

The mode of action of the three resistance genes used in this work are apparently different and might have contributed to make the resistance in the three-gene pyramid lines quite durable.

Phenotypic screening against BB not only revealed three gene positives (plants possessing *Xa21*, *xa13* and *xa5*) exhibiting excellent resistance to BB, but also observed that two gene positives possessing *Xa21* and *xa13* or *Xa21* and *xa5* were found to be resistant to BB. In the reports of Sundaram *et al.*, (2009), it was observed that triguna lines possessing *xa13* and *xa5* gene combination exhibited good resistance against BB disease along with *Xa21* and *xa13* combination. However, lines possessing *Xa21* and *xa5* combination exhibited longer lesion lengths but in our reports this combination too exhibited good levels of resistance.

Agro-morphological characterization of ten homozygous positive BC₁F₂ plants possessing all the three targeted genes in comparison with parents for the key agronomic traits resulted in identification of five superior plants *viz.*, NLR-87-5-1, NLR-87-16-11, NLR-87-6-15, NLR-87-9-24 and NLR-87-6-7 which were observed to be better than both the parents *viz.*, NLR34449 and ISM in terms of thousand grain weight (14.5-17.0 g), single plant yield (25.5-29.75

g) with fine grain type and complete panicle exertion (Table 2, Figure 2). However, no yield penalty was observed in the remaining lines and did not show any variation as compared to NLR34449 parent in terms of flowering duration, panicle exertion and yield parameters. Such observations were earlier reported by Pradhan *et al.*, (2015). These lines were forwarded to BC₁F₃ generation for further breeding programme.

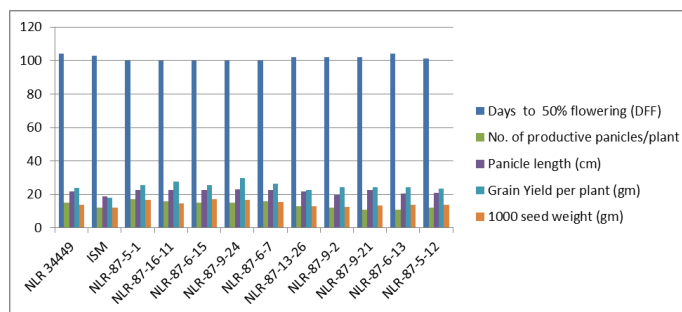


Figure 2: Graphical representation of agro-morphological characterization for yield and its component traits in BC₁F₁ population of the cross between NLR 34449 and ISM

Conclusion

The improved versions of Nellore mahsuri possessing BB and blast resistance, developed in the present study may offer a distinct advantage to farmers of NLR34449, whose fields are affected by both bacterial blight and blast. Further, Cultivation of

Table 2: Agro-morphological characterization for yield and its component traits in BC₁F₂ population of the cross between NLR34449 and ISM

S. No.	Plant identity	Days to 50% flowering (DFF)	Plant height (cm)	No. of productive panicles/plant	Panicle length (cm)	Grain Yield per plant (gm)	1000 seed weight (gm)	Grain type	Panicle exertion
1	NLR 34449	104	78	15	22	24	14	MS	FE
2	ISM	103	82	12	19	18	12	MS	PE
3	NLR-87-5-1	100	81	17	22.6	25.5	16.75	MS	FE
4	NLR-87-16-11	100	80	16	22.5	27.50	14.5	MS	FE
5	NLR-87-6-15	100	79	15	22.5	25.75	17.0	MS	FE
6	NLR-87-9-24	100	80	15	23.0	29.75	16.75	MS	FE
7	NLR-87-6-7	100	80	16	22.5	26.5	15.5	MS	FE
8	NLR-87-13-26	102	81	13	22	22.85	12.95	MS	FE
9	NLR-87-9-2	102	82	12	19.75	24.5	12.5	MS	FE
10	NLR-87-9-21	102	83	11	22.5	24.5	13.50	MS	FE
11	NLR-87-6-13	104	80	11	20.75	24.15	14.0	MS	FE
12	NLR-87-5-12	101	80	12	21	23.5	13.75	MS	FE

MS: Medium slender; FE: Fully Exerted



such improved backcross derived lines of NLR34449 possessing resistance against bacterial blight and blast could help to improve rice production in the disease endemic areas in many states of India, wherein fine-grain type varieties like NLR34449, Samba Mahsuri, HMT Sona etc. are preferred.

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