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Identification of Bacterial Blight Resistance Genes Introgressed Individuals in the Segregating Population of Rice †

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Abstract: Rice is the most consumed food crop around the globe. Bacterial blight (BB) caused by *Xanthomonas oryzae* pv *oryzae* (Xoo) is the most destructive bacterial disease in rice. The cross CB 87 R × (CB 87 R × IRBB 60) was screened for three BB resistance genes *Xa*21, *xa*13 and *xa*5 with the help of molecular markers revealed 15 individuals found to have resistance genes. The identified individuals with *Rf* gene were considered as an important criterion in the high yielding background, and the stabilized individuals could be used as genetic stocks for disease resistance breeding program in rice.

Keywords: hybrid rice; bacterial blight; gene introgression; marker assisted breeding

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1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops consumed worldwide. BB (bacterial blight) is deadliest bacterial disease caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) leads to severe yield reduction up to 80% in rice [1]. To overcome these yield losses, identification of tolerant/resistant germplasms/landraces sources and introgression of major governing resistance genes into the high yielding elite parental lines would be attractive to increase productivity [2]. Also, stacking of two or more genes into the single cultivar is an effective methodology to enhance the durability of the resistance genes. Marker assisted selection (MAS) is most widely used method to incorporate multiple resistance genes from donor parents into the breeder's interest breeding lines [3–8]. Most of the released hybrids/cultivars available in the markets are highly susceptible to rice BB and blast diseases. The most promising rice hybrid CORH 03 released by Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India, which is under large scale cultivation these areas, and it's became susceptible to BB over the period. The present study was aimed to intorogress and improve the agronomic performances of the parental line of released hybrid by employing marker assisted breeding (MAB).

2. Materials and Methods

Parents CB 87 R and IRBB 60 were used as recurrent and donor parents in this study, respectively. CB 87 R is the restorer parent of popular rice hybrid CORH 03, which is first non-aromatic and non-sticky rice hybrid. The parent IRBB 60 possess three BB resistance genes, of these two are recessive (*xa*5 and *xa*13) and another one is dominant in nature (*Xa*21). The hybrid (F_1) was generated by crossing between CB 87 R × IRBB 60, and the resistance allele governing individuals in the F_1 was confirmed by PCR (polymerase chain reaction) based molecular markers. The identified heterozygous F_1 individual plants for all three genes (*xa*5, *xa*13 and *Xa*21) were tagged and backcrossed with recurrent parent

CB 87 R to generate BC_1F_1. The BC_1F_1 individuals of the cross CB 87 R x (CB 87 R x IRBB 60) was screened for BB resistance genes with the help of foreground molecular markers. All these experiments were conducted at Department of Rice (11° N, 77° E, and 427 m above mean sea level, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India.

Fresh leaf tissues were collected from parents and their hybrids for genomic DNA extraction using CTAB (cetyltrimethylammonium bromide) method [9]. Two SSRs (RM 122 and RM 21) for *xa*5 and *Xa*21 genes, and one gene specific marker (xa13) for *xa*13 gene, were used in this study to tag the resistance genes in the studied materials. The PCR was carried out in thermal cycler and the protocol follows with an initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 1 min, annealing (for RM 122–55 °C for xa13–59 °C RM 21–55 °C) for 1 min, and primer extension at 72 °C for 1.30 min for 35 cycles, and the final extension at 72 °C for 7 min. The amplified PCR product (5 µL) was subjected to agarose gel electrophoresis, and bands were visualized using UV trans-illumination after ethidium bromide staining.

A *Xoo* strain was isolated from Department of rice, TNAU, Coimbatore, and was multiplied on PSA (peptone sucrose agar plates) followed by incubation for 48 h at 28 °C, and then 10 mL of distilled water added per slant to produce higher concentration of bacterial cells [10^8 to 10^9 colony-forming units (CFU)/mL]. Forty-eight BC_1F_1 individuals and their parents were inoculated the Xoo isolate by leaf clipping method when plants reached maximum of tillering/panicle emergence according to Kauffman et al. [10]. BB disease resistance reaction scoring was done 14 days after inoculation followed as standard evaluation system in 2011–2012 (SES 2011–2012).

3. Results

The total of 48 BC_1F_1 individuals from the cross CB 87 R × (CB 87 R × IRBB 60) was genotyped for BB resistance genes. Of these, 15 individuals were found to have resistance genes governed in different genes combinations (Figure 1). However, three BC_1F_1 individuals have all three genes in heterozygous conditions (*Xa*5*xa*5, *Xa*13*xa*13, *Xa*21*Xa*21). The same set of materials were also phenotyped for grain yield, some of genes introgressed individuals had higher single plant yield (21 to 25 g) than their original parental lines. Also, the identified heterozygous plants for BB genes were screened for presence of *Rf* genes. Genes introgressed resistant plants with *Rf* gene were selected in the high yielding background would further used for back crossing and selfing.

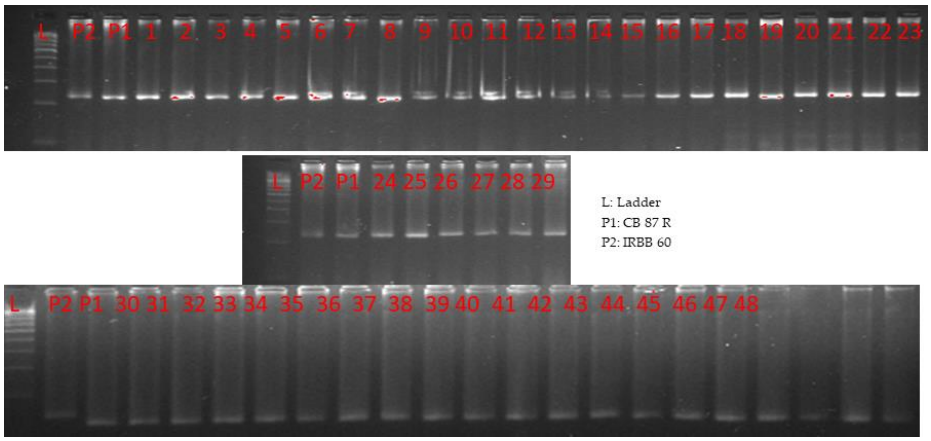

Figure 1. Identification of *xa5* bacterial blight resistance gene in BC_1F_1 cross of CB 87 R × (CB 87 R × IRBB 60)**.**

Table 1. Single plant grain yield of selected gene introgressed progenies of BC_1F_1 cross of CB 87 R × (CB 87 R × IRBB 60).

Plant No.	Single Plant Yield (g)
10	22.60
14	22.30
30	21.32
32	22.22
36	24.78

4. Discussion

A set of 48 individuals of BC_1F_1 hybrid CB 87 R × (CB 87 R × IRBB 60) screened and identified 15 individuals found to have different genes combinations through marker assisted foreground selection. Three out of 15 had all three genes in heterozygous combinations identified, and these were advanced next round of breeding cycles to stabilize these genes in homozygous conditions. The *Xa*21 gene and in combination with other genes introgressed individuals showed higher levels of tolerance than any of other combinations. Several studies have also been successfully introgressed/pyramided BB resistance genes into their parental lines PR36944-700 (TGMS) [11], PRR78 and KMR3 (restorers) and IR58025B and Pusa 6B (maintainers) [12,13], MTU 1010 [14], JGL1798 [15], and MR219 [16]. The identified promising genes introgrossed individuals in high yielding agronomic background will be further advanced and could be the potential resource to the breeders to use in their breeding programs.

5. Conclusions

The newly constructed genes introgressed individuals in this study will be serve as a base source to rice breeders in future to breed disease resistant cultivars to improve agricultural production.

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