chemistry proceedings

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Proceeding Paper

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

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† Presented at the 1st International Online Conference on Agriculture—Advances in Agricultural Science and Technology, 10–25 February 2022; Available online: https://iocag2022.sciforum.net/.

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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Citation: Govintharaj, P.; Manonmani, S.; Karthika, G.; Robin, S. Identification of Bacterial Blight Resistance Genes Introgressed Individuals in the Segregating Population of Rice. *Chem. Proc.* **2022**, *10*, 12. https://doi.org/10.3390/IOCAG2022-12243

Academic Editor: Daniel Tan

Published: 11 February 2022

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1. Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops consumed worldwide. BB (bacterial blight) is the deadliest bacterial disease caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) and leads to a severe yield reduction of up to 80% in rice [1]. To overcome these yield losses, the identification of tolerant/resistant germplasms/landraces sources and the introgression of the major governing resistance genes into the high-yielding elite parental lines would be attractive to increase productivity [2]. Moreover, the stacking of two or more genes into a single cultivar is an effective methodology for enhancing the durability of the resistance genes. Marker-assisted selection (MAS) is the most widely used method for the incorporation of multiple resistance genes from donor parents into the breeder's breeding lines of interest [3–8]. Most of the released hybrids/cultivars available on the market 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 in these areas, has recently become susceptible to BB. The present study was aimed at introgressing and improving the agronomic performances of the parental line of the released hybrid by employing marker-assisted breeding (MAB).

2. Materials and Methods

The 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 the popular rice hybrid, CORH 03, which is a non-aromatic and non-sticky rice hybrid. The parent, IRBB 60, possesses 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 through the crossing of CB 87 R × IRBB 60, and the resistance allele governing the individuals in the F_1 was confirmed by PCR (polymerase chain reaction)-based molecular markers. The identified heterozygous F_1 individual plants for all of the 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 × (CB 87 R × IRBB 60) were screened for BB resistance genes with the help of foreground molecular markers. All of these experiments were conducted at the 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 the CTAB (cetyltrimethylammonium bromide) method [9]. Two SSRs (RM 122 and RM 21) for *xa*5 and *Xa*21 genes, and one gene-specific marker (*xa*13) for the *xa*13 gene, were used in this study to tag the resistance genes in the studied materials. The PCR was carried out in a thermal cycler, and the protocol followed with an initial denaturation at 94 °C for 5 min, denaturation at 94 °C for 1 min, annealing (for RM 122–55 °C for *xa*13–59 °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 the bands were visualized using UV trans-illumination after ethidium bromide staining.

A *Xoo* strain was isolated from the 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 was added per slant to produce a 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 with the Xoo isolate by the leaf clipping method when the plants reached the maximum tillering/panicle emergence, according to Kauffman et al. [10]. BB disease resistance reaction scoring was conducted 14 days after inoculation, following the standard evaluation system in 2011–2012 (SES 2011–2012).

3. Results

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

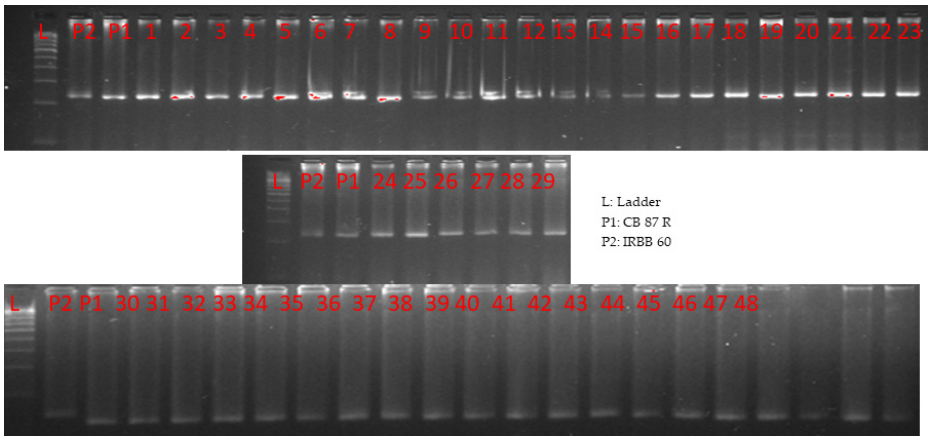

Figure 1. Identification of xa5 bacterial blight resistance gene in BC1F1 cross of CB 87 R × (CB 87 R × IRBB 60).

Table 1. Single plant grain yield of selected gene introgressed progenies of BC1F1 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 the BC_1F_1 hybrid CB 87 R × (CB 87 R × IRBB 60) screened and identified for 15 individuals were found to have different gene combinations through marker-assisted foreground selection. Three out of the 15 had all three genes in heterozygous combinations identified, and these were advanced in the next round of breeding cycles to stabilize these genes in homozygous conditions. The *Xa*21 gene and in combination with other gene-introgressed individuals, showed higher levels of tolerance than any of the other combinations. Several studies have also 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 gene-introgressed individuals in a high-yielding agronomic background will be further advanced and could be a potential resource for breeders to use in their breeding programs.

5. Conclusions

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

Author Contributions: Conceptualization, S.M., P.G., and S.R.; methodology, P.G.; software, P.G.; validation, S.M., P.G. and S.R.; formal analysis, P.G., G.K.; investigation, P.G.; resources, S.M., S.R.; data curation, P.G., S.M.; writing—original draft preparation, P.G., S.M., G.K.; writing—review and editing, P.G., S.M.; visualization, P.G., G.K.; supervision, S.M., S.R.; project administration, S.M.; funding acquisition, S.M., S.R. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: This research work is a part of M.Sc. thesis submitted to the Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu.

Conflicts of Interest: The authors declare no conflict of interest.

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