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Introgression of Bacterial Blight Resistance Genes (*Xa*21, *xa*13 and *xa*5) into CB 174 R, an Elite Restorer Line in Rice †

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Abstract: Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is one of the major diseases causing a severe yield reduction in rice-growing regions. One dominant (*Xa*21) and two recessive genes (*xa*13 and *xa*5) were introgressed into CB 174 R through marker-assisted breeding. The present study found three (*Xa*21 + *xa*13 + *xa*5) and two (*Xa*21 + *xa*13 or *Xa*21 + *xa*5 or *xa*5 + *xa*13) gene-introgressed combinations in the early segregated materials through foreground selection. The identified homozygous/heterozygous individuals were forwarded to the next cycles of breeding to fix homozygous conditions for all three genes with an improved agronomic performance background and, thus, could be used as a donor source for a future rice breeding program.

Keywords: marker-assisted selection; gene-specific marker; gene pyramiding

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1. Introduction

Rice (*Oryza sativa* L.) is an important staple cereal food crop for half of the world populations. Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) significantly reduces the yield of up to 80% in rice [1]. Till date, a total of 40 BB has been identified from wild, cultivated and mutant populations of rice [2]. Of these, BB resistance genes *Xa*3, *Xa*4, *Xa*7 and *Xa*21 have been extensively utilized by breeders in their breeding programs [3–7]. Breeding strategies include stacking of multiple resistance genes into the elite genetic background [8]. In this context, marker-assisted selection (MAS) is an efficient and cost effective approach along with precise phenotyping for disease-free cultivar development which has been proven by several rice researchers in the past [9,10]. The CORH 04 is a medium duration grain quality hybrid popular among farmers which was released by Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. However, hybrid CORH 04 was susceptible to BB disease in the rice-growing areas, resulting in a significant yield reduction. The CB 174 R is an elite restorer line which has been involved as a parent in several released high-yielding rice hybrids. An improvement of parental lines of the hybrid would be the best option to develop resistance against BB disease through MAS [11]. Therefore, the present study aimed to introgress the BB resistance genes to parental lines of the released rice hybrid through MAS.

2. Experiments

The experimental material CB 174 R is an elite restorer line of popular released rice hybrid CORH 4, used as a recurrent parent. The parent IRBB 60 with two recessive genes (*xa*5 and *xa*13) and one dominant gene (*Xa*21) was used as a donor. Two functional markers (*xa*5 and *xa*13) [12,13] and one set of SSR (simple sequence repeats) (RM 21 for *Xa*21 gene) [14] markers used to tag traits of interest. The hybrid F_1 generated by crossing CB 174 R and IRBB 60 was used. The BB resistance genes confirmed the F_1

plant along with a phenotypically desirable plant tagged and advanced to F_2 through self-fertilization breeding. A hundred and ten F_2 individual plants were screened for BB genes by employing gene-specific and SSR markers and phenotyped for BB isolate. A total of 54 out of 110 F_2 individual plants possessed all three or at least two BB resistance genes tagged with foreground selection and forwarded to $F_{2:3}$ through self-fertilization. All these field experiments were conducted at the Department of Rice (11° N, 77° E, and 427 m above mean sea level), Paddy Breeding Station (PBS), Tamil Nadu Agricultural University, Coimbatore, India.

Two grams of fresh leaf bits collected from 18-day-old seedlings of 110 F_2 individuals were used for genomic DNA (Deoxyribonucleic Acid) using CTAB (Cetyltrimethylammonium bromide) method as described by Doyle and Doyle [15]. The PCR (Polymerase chain reaction) was performed for two functional markers and one set of SSRs with an initial denaturation at 94 °C for 5 min 35 cycles of 1 min denaturation at 94 °C for 1 min annealing (for xa5–56 °C for xa13–59 °C RM 21–55 °C) and 1.30 min for primer extension at 72 °C, and the final extension at 72 °C for 7 min. The 5 µL PCR product was subjected to gel electrophoresis and then bands were visualized using UV trans-illumination after ethidium bromide staining. For, functional marker *xa*5, the PCR product was digested with *Bsr* 1 and bands were visualized the same as other markers.

One isolate of *Xoo*, prevalent in major rice growing areas, was isolated and multiplied on peptone sucrose agar plates and incubated for 48 h at 28 °C and then inoculum of the isolate into suspension by adding 10 mL of distilled water per slant to give a concentration of bacterial cells of about 10^8 to 10^9 colony-forming units (CFU)/mL. Hundred and ten F_2 individuals and their parents were artificially inoculated from the Xoo isolate when plants reached maximum of tillering as described earlier by Kauffman et al. [16]. Disease reaction scoring was performed 14 days after inoculation based on standard evaluation system in 2011–2012 (SES 2011–2012) (Table 1).

Table 1. SES scale for bacterial leaf blight (2011–2012).

S.No.	Score	Description (Affected Lesion Area)
1	0–1 (Resistant)	1–5% of leaf area affected
2	1–3 (Moderately Resistant)	6–12% of leaf area affected
3	3–5 (Moderately Susceptible)	13–25% of leaf area affected
4	5–7 (Susceptible)	26–50% of leaf area affected
5	7–9 (Highly Susceptible)	51–100% of leaf area affected

3. Results

The 110 F_2 individuals using foreground selection molecular markers led to identifying combinations of gene-introgressed individuals (Figure 1). None of the F_2 individuals showed homozygous resistance loci for all three genes. Though, a higher number of F_2 individuals in CB 174R × IRBB 60 identified having homozygous resistance for two loci (*Xa*21*Xa*21and *xa*13*xa*13) and also three F_2 individuals identified having two heterozygous resistance loci (*Xa*5*xa*5 and *Xa*21*xa*21). Furthermore, five F_2 individuals were identified in a heterozygous state in all three genes (*Xa*5*xa*5, *Xa*13*xa*13 and *Xa*21*Xa*21) and also two individuals were identified having heterozygous resistance for two loci (*Xa*5*xa*5 and *Xa*13*xa*13) and homozygous for another locus (*Xa*21*Xa*21). Based on the phenotypic score and resistance gene's combination, 54 F_2 individuals were selected, sealed, and advanced to the next generation ($F_{2:3}$). Nine gene-introgressed $F_{2:3}$ individuals showed a maximized yield potential compared to parents with recurrent background features (Table 2).

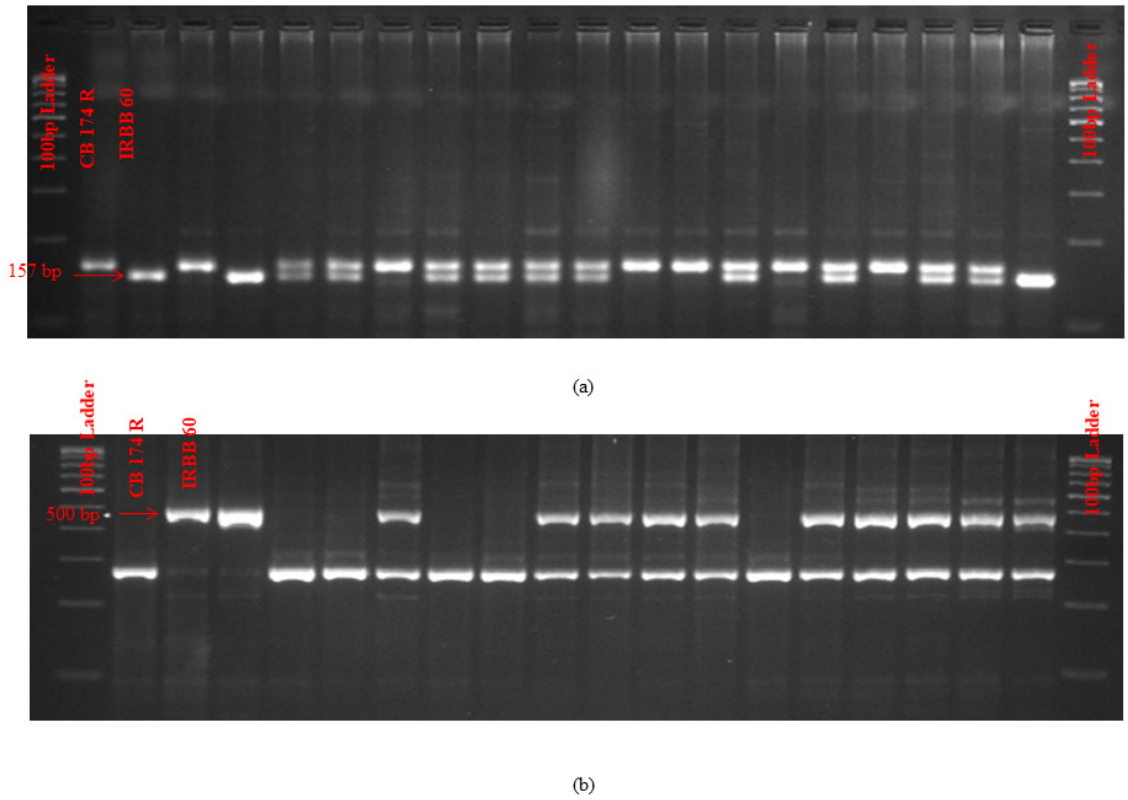

Figure 1. Gene tagging of F_2 population derived from cross between CB 174 R and IRBB 60 for functional marker (**a**) *xa*13, and (**b**) *Xa*21-linked bacterial blight resistance genes.

Table 2. Morphological characteristics of selected progenies of $F_{2:3}$ population of CB 174 R × IRBB60.

Plant. No	PH (cm)	NPT	PL (cm)	NG	TGW (g)	SPY (g)
1	115	16	25	102	21.90	35.5
2	105	14	23	192	24.05	34.5
3	116	15	27	198	18.52	41.0
4	119	12	26	141	23.62	33.0
5	115	17	20	137	21.07	37.0
6	105	19	21	129	23.4	32.5
7	94	22	20	167	22.18	32.0
8	115	17	14	95	22.09	31.5
9	100	17	21	253	24.70	48.5
CB 174 R	146.67	13	31.67	270.67	16.35	28.14
IRBB 60	88	11	27	124	13.60	22.90

Note: PH—plant height; NPT—number of productive tillers; PL—panicle length; NG—number of grains per panicle; TGW—thousand grain weight; SPY—single plant yield.

4. Discussion

A total of 54 out of 110 F_2 were individuals identified as having three/two gene combinations in this study. Of these, 42 individuals had the fertility restoration gene *Rf*4 characterized earlier in CB 174R × IRBB 60 by Govintharaj et al. [17]. We further found that five F_2 individuals were in the heterozygous state for all three genes (*Xa*5*xa*5, *Xa*13*xa*13 and *Xa*21*Xa*21), and also two individuals had the heterozygous resistance for two loci (*Xa*5*xa*5 and *Xa*13*xa*13) and homozygous for one locus (*Xa*21*Xa*21), along with fertility genes which were characterized earlier. The presence of *Xa*21 in the homozygote or heterozygote state in combination with other genes were found to have a higher level of resistance. Additionally, two recessive genes showed a higher level of resistance when they were in a homozygote (*xa*5*xa*5 and *xa*13*xa*13) rather than a heterozygote (*Xa*5*xa*5 and

*Xa*13*xa*13) condition. Similar to this study, Perumalsamy et al. [18] pyramided three BB resistance genes (*xa*5, *xa*13 and *Xa*21) using functional markers in rice. More than 80% of the F_2 individuals possessing an *Xa*21 + *xa*13 gene combination in this study showed a higher level of resistance. Several studies have shown a similar level of resistance (*Xa*21 + *xa*13) with gene-pyramided lines such as in Samba Mahsuri, PR106, Pusa Basmati 1, and IR 24, which could provide long-lasting resistance in India [8,19,20]. It has been stated that a broad spectrum of resistance was observed when multiple genes introgressed into an elite line rather than a single gene against BB resistance [21]. The identified different combinations of homozygous/heterozygous resistance plants F_2 with fertility restoration genes, and the subsequent $F_{2:3}$ families showed an improved agronomic performance which could be used as a donor parent for a future rice breeding program.

5. Conclusions

BB resistance genes identified in heterozygous and/or homozygous condition with superior agronomic performances of the studied breeding materials led to the use as a donor parent in BB resistance gene-introgression breeding.

Supplementary Materials: The poster presentation is available online at https://www.mdpi.com/article/10.3390/IECPS2020-08759/s1.

Author Contributions: S.M., P.G. and S.R. conceived and designed the experiments; P.G. performed the experiments; P.G. and S.M. analyzed the data; S.M. contributed reagents/materials/analysis tools; P.G., S.M. and G.K. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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Abbreviations

BB	Bacterial blight
MAS	Marker-assisted selection
SSR	Simple sequence repeats
DNA	Deoxyribonucleic Acid
CTAB	Cetyltrimethylammonium bromide
PCR	Polymerase chain reaction
CFU	Colony-forming unit
SES	Standard evaluation system

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