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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 disease caused severe yield reduction in the 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) genes introgressed combinations in the early segregated materials through foreground selection. The identified homozygous/heterozygous individuals forwarded to 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 future rice breeding programme.

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

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*), which is significantly reduces the yield up to 80% in rice [1]. Till date, a total of 40 BB have been identified from wild, cultivated and mutant population of rice [2]. Of these, BB resistance genes *Xa*3, *Xa*4, *Xa*7, and *Xa*21, have been extensively utilized by the breeders in their breeding programs [3–7]. Breeding strategies like 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 cultivars development which has been proved 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 grown areas resulted significant yield reduction. The CB 174 R is an elite restorer line have been involved as 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 was 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) is used as a donor. Two functional markers (xa 5 and xa 13) [12,13] and one 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. The BB resistance genes confirmed F_1 plant along with phenotypically desirable plant tagged and advanced to F_2 through self fertilization breeding. Hundred and ten F_2 individual plants screened for BB genes by employing gene-specific and SSR markers, and phenotyped for BB isolate. Fifty four out of 110 F_2 individual plant were possessed all three or two BB resistance genes tagged with foreground selection and forwarded to $F_{2:3}$ through self fertilization. All these field experiments were conducted at 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 days old seedlings of 110 F_2 individuals used for genomic DNA (Deoxyrhibonucleic Acid) using CTAB (Cetyltrimethylammonium bromide) method as described by Doyle and Doyle [15]. The PCR (Polymerase chain reaction) performed for two functional markers and one 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 subjected to gel electrophoresis and then bands 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 as same like 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 the Xoo isolate when plants reached maximum of tillering as described earlier by Kauffman et al. [16]. Disease reaction scoring was done 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 (Resistance)	1–5% of leaf area affected
2	1–3 (Moderately Resistance)	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 identify combinations of genes introgressed individuals (Figure 1). None of the F_2 individuals showed homozygous resistance loci for all three genes. Though, more 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, 5 F_2 individuals were identified in 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 other locus (*Xa*21*Xa*21). Based on phenotypic score and resistance gene's combination, 54 F_2 individuals were selected, selfed and advanced to next generation ($F_{2:3}$). Nine gene introgressed $F_{2:3}$ individuals showed maximized yield potential than parents with recurrent background features (Table 2).

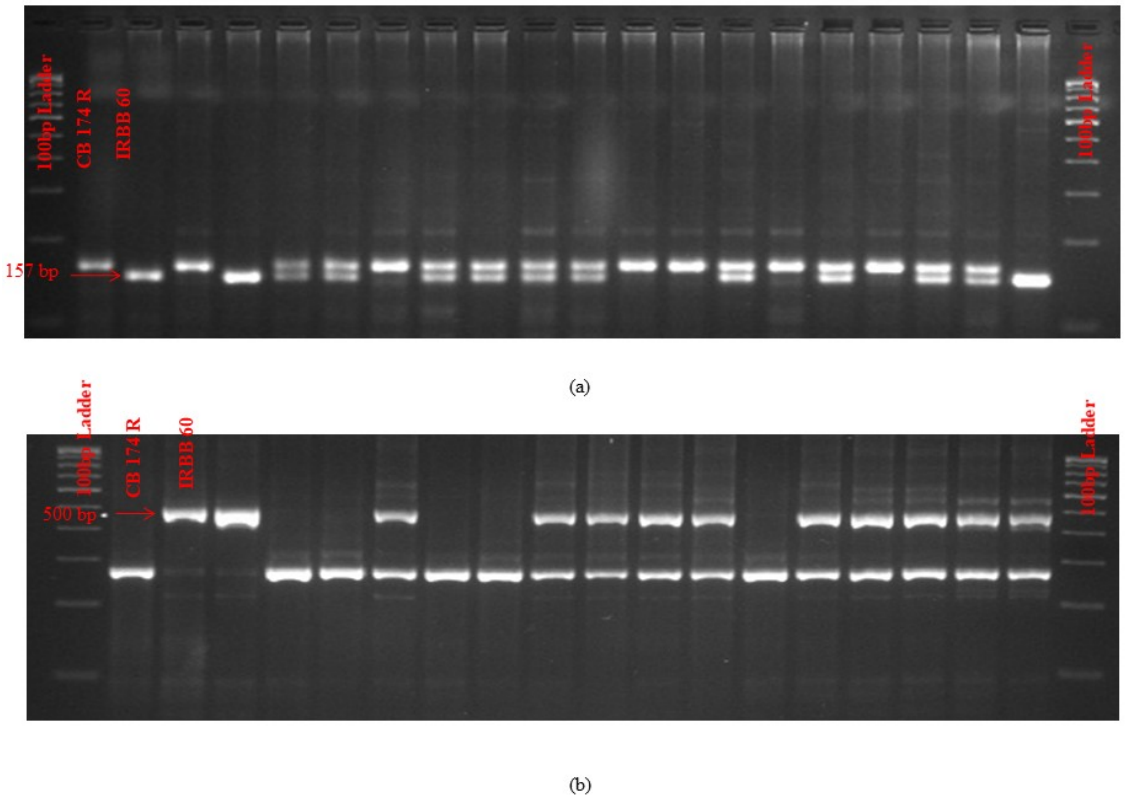

Figure 1. Gene tagging of F_2 population derived from cross between CB 174 R × IRBB 60 for functional marker (**a**) *xa*13, and (**b**) *Xa*21 linked bacterial blight resistance genes.

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

Fifty four out of 110 F_2 individuals identified as having three/two genes combination in this study. Of these, 42 individuals having fertility restoration gene *Rf*4 characterized earlier in CB 174R × IRBB 60 by Govintharaj et al. [17]. We further focused 5 F_2 individuals were in heterozygous state for all three genes (*Xa*5*xa*5, *Xa*13*xa*13 and *Xa*21*Xa*21), and also two individuals had 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. Presence of *Xa*21 in homozygote or heterozygote

state in combinations with other genes found to have higher level of resistance. Also, two recessive genes shown higher level of resistance when they were in homozygote (*xa*5*xa*5 and *xa*13*xa*13) than heterozygote (*Xa*5*xa*5 and *Xa*13*xa*13) condition. Similar to this study, Perumalsamy et al. [18] who have 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 possessed *Xa*21 + *xa*13 genes combination in this study showed higher level of resistance. Several studies have been showed same level of resistance (*Xa*21 + *xa*13) with gene-pyramided lines like in Samba Mahsuri, PR106, Pusa Basmati 1 and IR 24 could provide long-lasting resistance in India [19–21]. It has been stated that broad spectrum of resistance observed when multiple genes introgressed into a elite line rather than single gene against BB resistance [22]. 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 would be used as a donor parent for future rice breeding programme.

5. Conclusions

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

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

Abbreviations

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

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