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Introgression of three bacterial blight resistance genes into the background of 'high protein-Swarna' through marker assisted breeding

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Abstract
Rice (*Oryza sativa* L.) is the staple food of more than 70 percent of the Indian population. But, in recent decades, the crop has been severely impacted by adverse climatic effects and several biotic and abiotic stresses. The bacterial leaf blight disease is the main restriction among the biotic stresses that drop productivity in lowland ecology. The bacterial leaf blight disease is a solemn yield-limiting issue in the late maturing group of rice, which is further intensified by the application of a high dose of nitrogenous fertiliser for a higher yield that is favourable for this disease's advance. As a result, popular varieties lacking resistance to major diseases and pests should be settled for long-term production. Realising the importance of the problem, the present investigation was undertaken, and three bacterial blight resistance genes were transferred into the background of 'high protein-Swarna' using a marker-assisted backcross breeding approach. Further, in epiphytic conditions, bacterial leaf blight disease screening was performed to identify susceptible and resistant plant populations. Using gene-based SSR markers like RM13 for the *xa5* gene, *xa13* prom for the *xa13* gene, and PTA-248 for the *xa21* gene, foreground selection was done for BLB resistance in each substituent backcross population. Also, plants were selected and tagged based on their morpho-physiological characteristics that bore resemblance to the recipient parents. In the epiphytic condition, bacterial leaf blight disease screening was performed to identify susceptible and resistant plant populations. The plant populations, along with their parents, susceptible check (swarna), and resistance check (IRBB5, IRBB13, 1RBB21), were inoculated with highly virulent strains NR-Xoo-P-05 of *Xanthomonas oryzaepv. oryzae* (Xoo) at maximum tillering stage. The variety CRDHAN 800 recorded with all three resistant genes had an average protein content of 5.90 per cent and a mean plot yield of 600.45 g. The resistant line CR4169-52-1, which was claimed to have all three resistance genes, had a mean plot yield of 890.90 g and an average protein content of 9.61%. Similar to line CR4169-52-4, which also possessed all three resistance genes, a mean plot yield of 831.75 g, and an average protein content of 9.61%. Considering all the criterion lines CR4169-52-1 and CR4169-52-4 reported as better performers, they may be tested further.

Keywords: Bacterial blight resistance, foreground selection, gene pyramiding, marker-assisted breeding and rice

Introduction
Rice (*Oryza sativa L.*) is the staple in the diet of more than 70 per cent of the Indian population (United States Department of Agriculture 2019) [23]. This crop is the backbone of livelihood for millions of rural households and plays vital role in the country's food security, so the term "rice is life" is most appropriate in Indian context. Across the world, rice is cultivated in an area of 162.57 million hectares with a production of 499.0 million metric tonnes during 2018-19 (United States Department of Agriculture 2020) [22]. Approximately, 90 per cent of the world's rice is produced and consumed in Asia. Globally India is the second largest producer of rice after China (FAO 2019) [4]. In India, rice occupies an area of nearly 43.8 million hectares, with a total production of 177.6 million tons and productivity of 4,057 kg/ ha (FAOSTAT 2021) [5]. It is grown in almost all the states; however, the major rice producing states of the country during 2018-19 are West Bengal, Uttar Pradesh, Andhra Pradesh, Telangana, Punjab, Odisha, Chhattisgarh, Tamil Nadu and Bihar (FAOSTAT 2020).
In recent decades, the crop has been severely impacted by adverse climatic effects, which is reflected in the global rice cultivation output, particularly in India

. In the face of an alarming global population surge and resources such as land, labour, inputs, and chemicals, future production should be achieved in an eco-friendly way. As a result, popular varieties lacking resistance to major diseases and pests should be settled for long-term production. Several biotic and abiotic stresses are the key constraints for higher rice production in rain-fed ecological practises. The bacterial leaf blight disease is the main restriction among the biotic stresses that drop productivity in lowland ecology. The bacterial leaf blight disease is a solemn yield-limiting issue in the late maturing group of rice, which is further intensified by the application of a high dose of nitrogenous fertiliser for a higher yield that is favourable for this disease's advance. The yield losses due to this disease generally vary from 20% to 40% (Sonti 1998) [20], but decrease the crop yield up to 50% (Khush *et al.* 1989) [11] or even up to 80% (Singh *et al.* 1977) [18] in Asia. Worldwide, a total of 45 BLB resistance genes have been acknowledged from various sources. Several leading donor lines with diverse resistance genes are being used in the BLB resistance varietal improvement research programmes. The resistance genes, specifically *xa4*, *xa5*, *xa7*, *xa13*, and *xa21*, have been widely exploited in BLB resistance advance programmes (Nayak *et al.* 2015, Pradhan *et al.* 2022 and Singh *et al.* 2001) [12, 15, 19]. Appropriate mixtures of BLB resistance genes from a variety of backgrounds are reported to be operative in contradiction to the pathogen isolates, though huge acreage and the long-term growth of rare varieties with single resistance genes may break down the pathogen resistance. The resistance breakdown can be slowed by pyramiding the appropriate BLB resistance genes into the popular rice cultivars. It is tough to transfer multiple resistance genes simultaneously through traditional improvement programmes.

Recent studies have reported several closely related molecular markers for use in locating the BLB resistance genes, including two and three resistance genes, in a plant. When two or more resistance genes are combined in a useful way, the mutation rate in pathogens for virulence is significantly lower than when a cultivar just possesses a single resistance gene. Strong and broad-spectrum pathogen resistance is provided by several resistance genes pyramiding in a single genetic environment. There are numerous closely related genetic markers for BLB resistance genes that have been successfully positioned through the use of marker-assisted breeding.

Realizing the importance of the problem, the present investigation was undertaken and three bacterial blight resistance genes were transferred in the background of 'high protein-Swarna' using a marker-assisted backcross breeding approach. Further, in epiphytic condition, bacterial leaf blight disease screening was performed to identify susceptible and resistant plant populations.

Materials and Methods

Plant material used in the experiment

The parents CR 2830-PLS-17 or CR 2830-PLS-156 (high protein Swarna lines) and CR Dhan 800 (Swarna with three bacterial blight resistance genes, *xa5, xa13,* and *xa21*) used for crossing were grown separately in a crossing block during the 2017 *Kharif* season. Each parent's female and male line seeds were planted separately in small pots. To assure constant flower availability for producing enough crossed seeds, three staggered sowings of the parents were made at a ten-day interval.

In *kharif* 2018, the BC_1F_1 population developed from a cross of CR 2830-PLS-17 or CR 2830-PLS-156 (high protein Swarna lines) and CR Dhan 800 (Swarna with three bacterial blight resistance genes, *xa5*, *xa13*, and *xa21*) rice varieties was advanced to the BC_1F_2 generation along with their parents, the BLB susceptible high yielding Swarna rice variety, and checks IRBB5, IRBB13, and IRBB21, and screening was done for the presence of the genes *xa5, xa13*, and *xa21*. Resistant plants were selected and tagged based on their morpho-physiological characteristics that bore resemblance to their parents. Validation of presence of BLB resistant genes *xa5, xa13*, and *xa21* was carried out with the help of tightly linked, flanking, or gene-based SSR markers. Foreground selection for BLB resistance of genes *xa5, xa13*, and *xa21* were carried out by linked SSR markers. The positive plants of BC_1F_{2s} having three resistant genes (*xa5, xa13*, and *xa21*) from two crosses were selected and tagged. The positive plants of BC_1F_{2s} were selfed to obtain BC_1F_3 generation seeds.

BC_1F_3 generations and their parents from two crosses were grown during the Rabi (offseason) season in December 2021–22 on nursery beds and later transplanted into the field following randomized block design. Foreground selection or validation for BLB resistant genes was performed using gene-based SSR markers.

Molecular characterization for bacterial leaf blight resistance genes

Isolation of genomic DNA

Tissues were homogenized using liquid nitrogen in a mortar and pestle, followed by the isolation of genomic DNA using the CTAB (Cetyl Trimethyl Ammonium Bromide) method (Doyle and Doyle 1987) [3] with some minor modifications.

Quantification of the purified DNA

Quantification of extracted DNA samples was done through spectro-photometric analysis at A260/A280 nm. For pure DNA, a ratio of 1.8–2.0 is considered appropriate. Further integrity of DNA was examined in 0.8% agarose gel electrophoresis by visualizing under UV gel documentation. The 4µl 6X DNA loading dye along with 2µl isolated genomic DNA were loaded in a 0.8% agarose gel stained with 5µl of ETBR (Ethidium Bromide). A known quantity of uncut lambda DNA was included as standard to recheck the quality and quantity. Gel electrophoresis was run at 80 volts for 45 minutes.

DNA markers used in the study

To ascertain the introgression of BLB genes, SSR markers RM13 (linked to *xa5* gene) Xa-13 prom (linked to *xa13* gene) and pTA248 (linked to *xa21* gene) were used as described by Huang *et al.* 1997 [8] (Table 1). Homozygous lines for different gene combinations were identified.

Table 1: List of BLB resistance genes and SSR markers used for foreground selection

Marker	Primer sequence	Annealing temperature (°C)
pTA-248 (STS)	F-AGACGCGGAAGGGTGGTTCCCGGA	55
	R-AGACGCGGTAATCGAAGATGAAA	
Xa13prom (SSR)	F-GGCCATGGCTCAGTGTTTAT	55
	R-GAGCTCCAGCTCTCCAAATG	
RM 13	F-TCCAACATGGCAAGAGACAG	55
	R-GGTGGCATTCGATTCCAG	

Polymerase chain reaction (PCR)

PCR amplification reactions were standardized for dNTP concentration, primer annealing temperature, concentration of primer, concentration of template DNA, and Taq DNA polymerase. Polymerase chain reaction analysis was carried out with a mixture containing 50ng of template DNA, 1 mM dNTPs, 1 µl of forward and reverse primers, 10X PCR buffer, and 1 unit/µl of Taq polymerase. The template DNA was initially denatured at 94 °C for 4 min, then each cycle consisted of 30 sec of denaturation at 94 °C, 30 sec of primer annealing at 55 °C, and 1 min of primer extension at 72 °C. A total of 38 cycles of PCR amplification were carried out. The amplified PCR products were resolved in 3% and 1.5% agarose gel electrophoresis at 110 volts for 2.5 hours and visualized after staining with ethidium bromide under a UV gel documentation unit using software Alpha imager.

Screening for bacterial leaf blight resistant plants

In the epiphytic condition, bacterial leaf blight disease screening was carried out. After 45 days of transplanting, the extremely virulent *Xanthomonas oryzae*pv. *oryzae* (Xoo) strains NR-Xoo-P-05 were inoculated using the leaf clipping method (Ke Y *et al.* 2017) [10] into the derived plant populations along with the parents, susceptibility check (swarna), and resistance check (IRBB5, IRBB13, and 1RBB21). The inoculums, at a final concentration of approximately 10^9 cells/ ml, were prepared by suspending bacteria in sterile distilled water, and plant inoculation was carried out by clipping the tip (about 1 to 2 cm) of the fully expanded uppermost leaf with scissors after dipping into the inoculum. As symptoms start to manifest after 14–21 days following inoculation, the disease reaction is measured using a standard evaluation system, abbreviated as SES (1980) [21], created at the International Rice Research Institute, Philippines (Table 2). Five leaves per plant were taken for scoring purposes from each progeny. The 1–9 scale is classified into six classes (coded as 0, 1, 3, 5, 7, and 9), inferring 0–1 as resistant (R), 3–5 as moderately resistant (MR), and 7–9 as susceptible (S). Observations on the severity of the BLB infection in each population were recorded.

Table 2: Scale for bacterial leaf blight disease

Infection (%)	Score	Host response
0%	0	Highly resistant (HR)
> 1-10%	1	Resistant (R)
> 10-30%	3	Moderately resistant (MR)
> 30-50%	5	Moderately susceptible (MS)
> 50-75%	7	Susceptible (S)
> 75-100%	9	Highly susceptible (HS)

Estimation of grain protein using NIR spectroscopy

The grain protein content (GPC) of selected introgressed lines possessing resistant gene combinations after molecular screening was estimated using NIR spectroscopy (Bagchi *et al.* 2015) [1]. For analysis using NIR spectroscopy Brown rice samples (10–15 g) were prepared through a rice huller (Satake Corporation, Japan), and after cleaning, they were immediately used for analysis with NIRS. A small cup (size: inner diameter 66 mm and height 25 mm) was used for scanning the sample with full spectrum (400–2500 nm), taking about 15 g of each sample. NIR analyses were accomplished for crude protein.

Results

Xanthomonas oryzaepv oryzae, which causes rice bacterial blight, is one of the most severe diseases of rice among the biotic stresses. Three target genes had polymorphism between the parents, according to a survey using the gene-specific markers pTA248; Xa13prom; and RM 13 (linked to *xa21*, *xa13*, and *xa5* respectively). From the BC_1F_1 stage onwards, foreground selection was used at each stage to select the plants that had resistance alleles of the three target genes, and only progenies with the resistance alleles were advanced to the next generation. Figures 1 to 3 represent the banding pattern of different genotypes for gens *xa5*, *xa13*, and *xa21*, respectively. Plants homozygous for three and two gene combinations were found in the BC_1F_3 generation, and the combinations acquired are as follows: four plants had the *xa5+xa13* combination, ten had the *xa5* + *xa21* combination and four had all three gene combinations (Table 3).

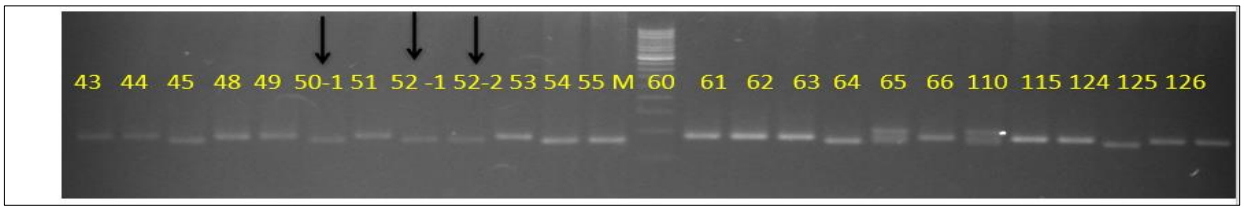

Fig 1: Foreground selection of BC_1F_3 plants for *xa5* through gene-linked markers. The numbers shown on top of the gel represent the BC_1F_3 plant numbers. M, molecular weight marker (50-bp ladder); A, Swarnanjali, CR DHAN 411 (recipient parent); B, CR DHAN 800 (donor parent for *xa5*). Arrow shows lines possessing the *xa5* gene, i.e., improved bacterial blight lines namely CR4169-42-2-40-1, CR4169-50-1, CR4169-52-1, and CR4177-4-3-235-1.

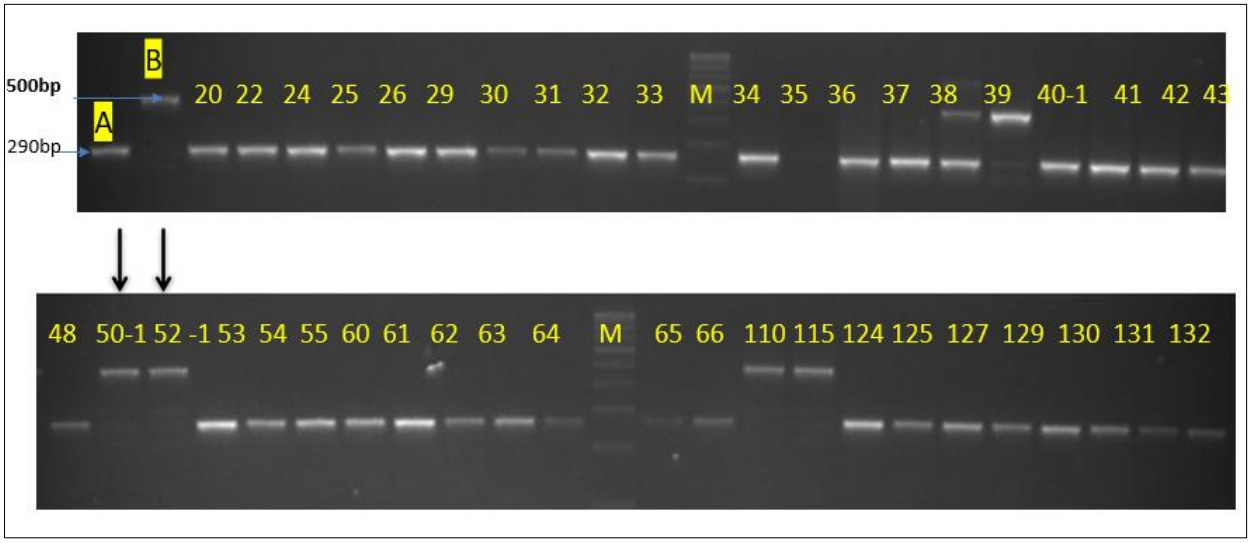

Fig 2: Foreground selection of BC_1F_3 plants for *xa13* through gene-linked markers. The numbers shown on top of the gel represent the BC_1F_3 plant numbers. M, molecular weight marker (100-bp ladder); A, Swarnanjali, CR DHAN 411 (recipient parent); B, CR DHAN 800 (donor parent for *xa13*). Arrow shows lines possessing the *xa13* gene, i.e., improved bacterial blight lines namely CR4169-50-1 and CR4169-52-1.

Fig 3: Foreground selection of BC1F 3 plants for *xa21* through gene-linked markers. The numbers shown on top of the gel represent the BC1F3 plant numbers. M, molecular weight marker (1Kb ladder); A, Swarnanjali, CR DHAN 411 (recipient parent), B, CR DHAN 800 (donor parent for *xa21*). Arrow shows lines possessing the *xa21* gene, i.e., improved bacterial blight lines namely CR4169-42-2-40-1, CR4169-52-1, CR4169-52-2 and CR4169-18-3-166-1.

In the epiphytic condition, bacterial leaf blight disease screening was carried out in *Rabi* 2021 and *kharif* 2021. After 45 days of transplanting, the extremely virulent Xanthomonas *oryzae*pv. *Oryzae* (Xoo) strains NR-Xoo-P-05 were inoculated using the leaf clipping method. Observations on the severity of the BLB infection in each population were recorded (Figure 4) and classification of line was done by six classes (coded as 0, 1, 3, 5, 7, and 9), inferring 0–1 as resistant (R), 3–5 as moderately resistant (MR), and 7–9 as susceptible (S) (Table 3). Bioassays against Xoo isolates validated the donor's (CR Dhan 800) and recurrent parent's (CR Dhan 411) resistant and susceptible reactivity, with lesser lesion lengths (0.54–1.70 cm) on CR Dhan 800 and greater lesion lengths (13.3–14.48 cm) on CR Dhan 411.

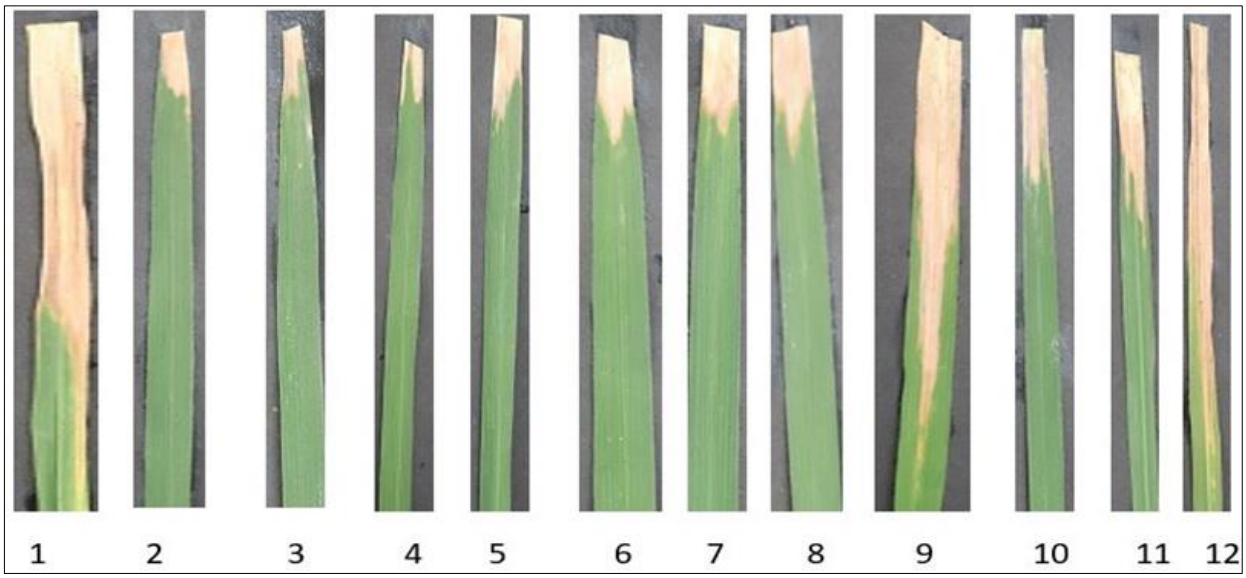

Fig 4: Bioassay using *xoo* isolates. The numbers 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 represent the recipient parent (SWARNANJALI), CR DHAN 800 (donor for BB resistance), improved BLB resistance lines, and susceptible BLB lines, that is, CR 4169-52-1, CR 4169-52-2, CR 4169-42-2-40-1, CR 4169-50-1, CR 4177-4-3-235-1, and CR 4169-1, respectively.

Using NIRS from the seeds that were gathered, protein estimation of the chosen plants was done, and it was discovered that it ranged from 9.61 to 11.45% (Table 3). The variety CRDHAN 800 recorded with all three resistant genes had an average protein content of 5.90 per cent and a mean plot yield of 600.45 g. The resistant line CR4169-52-1, which was claimed to have all three resistance genes, had a mean plot yield of 890.90 g and an average protein content of 9.61%. Similar to line CR4169-52-4, which also possessed all three resistance genes, a mean plot yield of 831.75 g, and an average protein content of 9.61%.

Table 3. Resistant and susceptible bacterial blight lines (based on SSR marker data and BLB scoring) with grain protein content and yield

Variety name	SSR data	RB-21 BLB Scoring	KH-21 BLB Scoring	RB-21 Protein (%)	KH-21 Protein (%)	Avg Protein (%)	RB-21 Plot yield (g)	KH-21 plot yield (g)	Avg plot yield (g)
PLS-17	**S**	7 and 9	7 and 9	7.46	9.12	8.29	850.90	998.8	924.85
SWARNA	**S**	7 and 9	7 and 9	5.98	6.04	6.01	644.30	624.8	634.55
CRDHAN 800	*xa5, xa13* and *xa21*	5	5	5.92	5.88	5.90	606.90	594	600.45
CR4169-22-3-17-1	*xa5* and *xa21*	5	7 and 9	6.52	10.74	8.63	677.43	1069.2	873.32
CR4169-22-3-17-2	*xa5* and *xa21*	5	7 and 9	6.17	10.5	8.34	646.59	629.2	637.90
CR4169-42-2-40-1	*xa5* and *xa21*	7 and 9	5	12.65	13.41	13.03	815.43	1368.4	1091.92
CR4169-42-2-40-2	*xa5* and *xa21*	7 and 9	5	12.73	13.35	13.04	807.00	800.8	803.90
CR4169-50-1	*xa5* and *xa13*	5	5	13.99	16.74	15.37	789.32	990	889.66
CR4169--50-2	*xa5* and *xa13*	5	5	12.58	16.12	14.35	760.00	761.2	760.60
CR4169-52-1	*xa5, xa13* and *xa21*	5	5	8.43	10.78	9.61	787.40	994.4	890.90
CR4169-52-2	*xa5, xa13* and *xa21*	5	5	7.06	9.85	8.46	756.00	743.6	749.80
CR4169-29-78-1	*xa5* and *xa21*	5	5	9.23	11.06	10.15	754.40	796.4	775.40
CR4169-29-78-1	*xa5* and *xa21*	5	5	7.96	10.09	9.03	785.70	1157.2	971.45
CR4169-29-79	*xa5*	3	7 and 9	7.66	9.18	8.42	503.00	347.6	425.30
CR4169-1-80	*xa5*	3	5	7.59	12.68	10.14	621.70	646.8	634.25
CR4169-16-109	*xa5*	5	5	8.77	12.59	10.68	542.40	554.4	548.40
CR4169-52-1-110	*xa5*	3	5	9.38	11.46	10.42	841.60	844.8	843.20
CR4169-44-1-126	*xa5*	5	7 and 9	7.68	11.53	9.61	567.80	563.2	565.50
CR4169-8-3-128	*xa5*	3	5	8.45	11.57	10.01	698.50	721.6	710.05
CR4169-42-2-140	*xa5*	3	5	9.94	13.58	11.76	765.70	787.6	776.65
CR4169-18-3-166-1	*xa5* and *xa13*	3	5	9.96	14.51	12.24	877.40	893.2	885.30
CR4169-25-1-168	*xa5*	3	7 and 9	6.47	9.3	7.89	1098.10	1245.2	1171.65
CR4169-13-1-169	*xa5*	3	7 and 9	8.64	8.72	8.68	953.20	1157.2	1055.20
CR4169-51-1-170	*xa5*	3	7 and 9	5.83	8.87	7.35	876.60	1130.8	1003.70
CR4169-10-2-189	*xa5*	3	7 and 9	7.76	10.78	9.27	888.90	1069.2	979.05
CR4169-42-1-190	*xa5*	3	5	7.65	11.15	9.40	532.80	448.8	490.80
CR4169-52-4	*xa5, xa13* and *xa21*	5	5	8.43	10.78	9.61	765.90	897.6	831.75
CR4169-52-5	*xa5, xa13* and *xa21*	5	7 and 9	7.87	10.54	9.21	712.40	673.2	692.80
CR-4177-4-135	*xa5*	5	7 and 9	9.54	11.79	10.67	1123.00	1029.6	1076.30
CR-4177-11-136-1	*xa5* and *xa21*	3	5	8.9	12.97	10.94	654.00	594	624.00
CR-4177-11-136-2	*xa5* and *xa21*	3	5	8.22	13.05	10.64	578.40	629.2	603.80
CR-4177-4-213	*xa5*	5	5	8.98	13.78	11.38	687.20	655.6	671.40

CR-4177-11-214	*xa5*	3	5	6.7	10.72	8.71	709.20	655.6	682.40
CR-4177-18-2-218	*xa5*	5	5	8.43	12.86	10.65	466.00	316.8	391.40
CR-4177-4-3-235-1	*xa5* and *xa21*	5	5	9.21	14.58	11.90	745.00	893.2	819.10
CR-4177-4-3-235-2	*xa5* and *xa21*	5	5	8.94	14.6	11.77	627.00	686.4	656.70
CR-4177-4-3-236	*xa5*	5	5	10.11	12.2	11.16	722.00	743.6	732.80
CR-4177-2-243	*xa5* and *xa13*	3	5	9.77	10.57	10.17	701.00	629.2	665.10

Discussion

Xanthomonas oryzaepv. *Oryzae*, which causes rice bacterial blight, is one of the most severe diseases of rice among the biotic stresses. Bacterial blight causes yield losses of up to 70% annually, which could fulfill the annual consumption of 60 million people (Parker *et al*. 2008) [14], and also affects rice quality (Goto 1992) [7]. Singh *et al*. 1977 and Ou 1985 [18, 13], have reported that rice yield losses in severely infected fields by bacterial blight generally range from 20 to 30%, and in some areas are reportedly up to 80%.

Three target genes had polymorphism between the parents, according to a survey using the gene-specific markers pTA248; Xa13prom; and RM 13 (linked to *xa21*, *xa13*, and *xa5* respectively). From the BC_1F_1 stage onwards, foreground selection was used at each stage to select the plants that had resistance alleles of the three target genes, and only progenies with the resistance alleles were advanced to the next generation. Figures 1 to 3 represent the banding pattern of different genotypes for gens *xa5*, *xa13*, and *xa21*, respectively. Plants homozygous for three and two gene combinations were found in the BC_1F_3 generation, and the combinations acquired are as follows: four plants had the *xa5+xa13* combination, ten had the *xa5* + *xa21* combination and four had all three gene combinations (Table 3). These results are in accordance with the findings of Basavaraj *et al*. 2010 and Sakthivel *et al*. 2017 [2, 16]. The results of screening against *Xoo* isolates indicate that all of the pyramid lines performed better than the parent CR Dhan 800. The three pyramid lines demonstrated significant levels of resistance compared to parent CR Dhan 800.

In the epiphytic condition, bacterial leaf blight disease screening was carried out, the extremely virulent Xanthomonas *oryzae*pv. *oryzae* (Xoo) strains NR-Xoo-P-05 were inoculated. Observations on the severity of the BLB infection in each population were recorded (Figure 4) and classification of line was done by six classes (coded as 0, 1, 3, 5, 7, and 9), inferring 0–1 as resistant (R), 3–5 as moderately resistant (MR), and 7–9 as susceptible (S) (Table 3). Bioassays against Xoo isolates validated the donor's (CR Dhan 800) and recurrent parent's (CR Dhan 411) resistant and susceptible reactivity, with lesser lesion lengths (0.54–1.70 cm) on CR Dhan 800 and greater lesion lengths (13.3–14.48 cm) on CR Dhan 411. The results of screening against Xoo isolates indicate that all of the pyramid lines performed better than the parent CR Dhan 800. The three pyramid lines demonstrated significant levels of resistance compared to parent CR Dhan 800.

Using NIRS from the seeds that were gathered, protein estimation of the chosen plants was done, and it was discovered that it ranged from 9.61 to 11.45% (Table 3). Plants with three BLB gene combinations and bioassay results indicating resistant protein content had a protein content of 9.61%. These results are in agreement with the findings of Joseph *et al*. 2004 and Salgotra *et al*. 2012 [9].

The variety CRDHAN 800 recorded with all three resistant genes had an average protein content of 5.90 per cent and a mean plot yield of 600.45 g. The resistant line CR4169-52-1, which was claimed to have all three resistance genes, had a mean plot yield of 890.90 g and an average protein content of 9.61%. Similar to line CR4169-52-4, which also possessed all three resistance genes, a mean plot yield of 831.75 g, and an average protein content of 9.61%.

Conclusion

The variety CRDHAN 800 recorded with all three resistant genes had an average protein content of 5.90 per cent and a mean plot yield of 600.45 g. The resistant line CR4169-52-1, which was claimed to have all three resistance genes, had a mean plot yield of 890.90 g and an average protein content of 9.61%. Similar to line CR4169-52-4, which also possessed all three resistance genes, a mean plot yield of 831.75 g, and an average protein content of 9.61%. Considering all the criterion lines CR4169-52-1 and CR4169-52-4 reported as better performers, they may be tested further.

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