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Incorporation of Two Bacterial Blight Resistance Genes into the Popular Rice Variety, Ranidhan through Marker-Assisted Breeding

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Abstract: The bacterial blight (BB) disease of rice is a major disease that reduces yield heavily in susceptible varieties. Ranidhan is a late maturing popular rice variety but shows high susceptibility to the disease. Two BB resistance genes were transferred into the variety through a marker-assisted backcross breeding approach. Tightly linked molecular markers were deployed to track the BB resistance genes in the plants carrying the target genes in each backcross generation. Foreground screening detected 17, 16 and 15 progenies to carry the 3 BB resistance genes in BC_1F_1, BC_2F_1 and BC_3F_1 generations, respectively. The selected BC_3F_1 plant was selfed and three different combinations of BB resistance genes were tracked in homozygous state in seven BC_3F_2 plants. The pyramided lines carrying three resistance genes in homozygous conditions were evaluated for BB disease resistance by inoculating with eight virulent *Xoo* strains. Five pyramided lines carrying two resistance gene combinations (*Xa21+xa13* and *Xa21+xa5*) exhibited enhanced resistance against the BB pathogens. The disease resistance was in the order of *Xa21+xa5* < *Xa21+xa13* < *xa13+xa5* gene combinations in conferring the resistance. The developed pyramided lines were similar to the recipient parent for the majority of the important agro-morphologic and grain quality traits.

Keywords: bacterial blight disease; foreground selection; resistance gene pyramiding; marker-assisted breeding; rice

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1. Introduction

Rice, the queen of cereals, serves as food and fodder for millions of people and domesticated animals around the world. The importance of the crop in India is not only as a staple food but also taken as a ceremonial crop [1]. Its dietary composition includes carbohydrates, proteins, oils, vitamins, antioxidants, fiber, minerals and a variety of plant chemicals [2]. Rice cultivation is possible in high elevations as well asbelow sea level due to its wide adaptability. It is cultivated nearly 45% as a rainfed crop out of a total of 160 mha of rice land in the world [3]. Contributing to the livelihood of about 400 crores of people constituting nearly 55% of the world population, rice generates nearly $206 billion annually, which is about 17% of the total value of the crop [4]. The crop is highly affected by adverse climatic effects in recent years, which is reflected in the production from the rainfed rice cultivation in the world in general and in India in particular [5]. At the juncture of an alarming population increase and resources such as land, labor, inputs and chemicals, future production should be obtained in an eco-friendly manner [6,7]. Therefore,

popular varieties deficient in resistance to major diseases and pests should be developed for sustainable production. In this investigation, the improvement of BB resistance and grain yield were targeted in rice.

Many biotic and abiotic stresses are the main constraints for higher rice production in rainfed ecology. BB is the major constraint amongst the biotic stresses in reducing productivity in low land ecology. The ecology covers about 15 mha in India, of which >80% are located in the eastern states of the country [8]. BB disease is a serious yield-limiting factor in the late maturing group of rice which is further aggravated by the application of a high dose of nitrogenous fertilizer for higher yield that is favorable for this disease development. The yield losses due to this disease normally vary from 20% to 40% [9], but reduce the crop yield up to 50% [10] or even up to 80% in Asia [11].

Globally, a total of 45 BB resistance genes have been identified from diverse sources [12]. Many elite donor lines with different resistance genes are being used in the BB resistance improvement programs [13]. The resistance genes, namely *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21*, have been extensively utilized in BB resistance improvement programs [14–16]. Suitable combinations of BB resistance genes in a variety of backgrounds are reported to be effective against the pathogen isolates. However, large acreage and long-term growth of few varieties with single resistance genes may break down the pathogen resistance. The resistance breakdown can be delayed by pyramiding suitable BB resistance genes into rice cultivars. It is difficult to transfer multiple resistance genes simultaneously through classical improvement programs. Recently, many closely linked molecular markers are reported for use in the BB resistance genes detection carrying two and three resistance genes together in a plant. The mutation in pathogen for virulence to two or more resistance genes in a suitable combination is much lower than for a single resistance gene in a cultivar. Multiple resistance gene pyramiding in a single genetic background provides a durable and broad-spectrum resistance against pathogens. Many closely linked molecular markers are available for BB resistance genes and are successfully deployed through the marker-assisted breeding approach [17–29]. The development of pyramided lines carrying two BB resistance genes, *xa13+Xa21* and *xa5+Xa21* through marker-assisted selection in the popular rice variety, Ranidhan is reported here. The results indicated an increased resistance to the BB disease against the eight *Xoo* strains populations.

2. Materials and Methods

2.1. Plant Materials Used in the Breeding Program

The elite parent, CR Dhan 800 carrying 3 resistance genes *Xa21*, *xa13* and *xa5* in the Swarna variety background for BB resistance was used as the male donor parent in the hybridization program. The recurrent parent, Ranidhan, is a late maturing variety but is highly susceptible to BB disease. The parental lines were obtained from the gene bank of the National Rice Research Institute (NRRI), Cuttack, India. Ranidhan, the recipient parent, was crossed with the donor variety, CR Dhan 800, during the wet season of 2017 as per the marker-assisted breeding scheme (Figure 1). A true F_1 plant developed during the previous wet season was backcrossed with the recipient parent during the dry season of 2018 for the production of BC_1F_1 seeds. Three BB resistance genes were tracked in the BC_1F_1 plants using the closely linked markers (Table 1). BC_1F_1 plant with more similarity with recipient variety was hybridized to produce BC_2F_1 seeds during the wet season of 2018. BC_2F_1 generation plants were developed during the dry season of 2019. BC_2F_1 plant with more similarity with a recurrent parent was hybridized to get BC_3F_1 seeds. Similarly, BC_3F_1 plants were developed during the wet season of 2019. BC_3F_2 generation progenies were produced from the selected foreground positive plants during the dry season of 2020. Foreground selection was performed in BC_3F_2 progenies to select the lines carrying the suitable target gene combinations. Evaluation of the pyramided and parental lines for yield, agro-morphologic and grain quality traits were conducted during the wet seasons of 2020 and 2021.

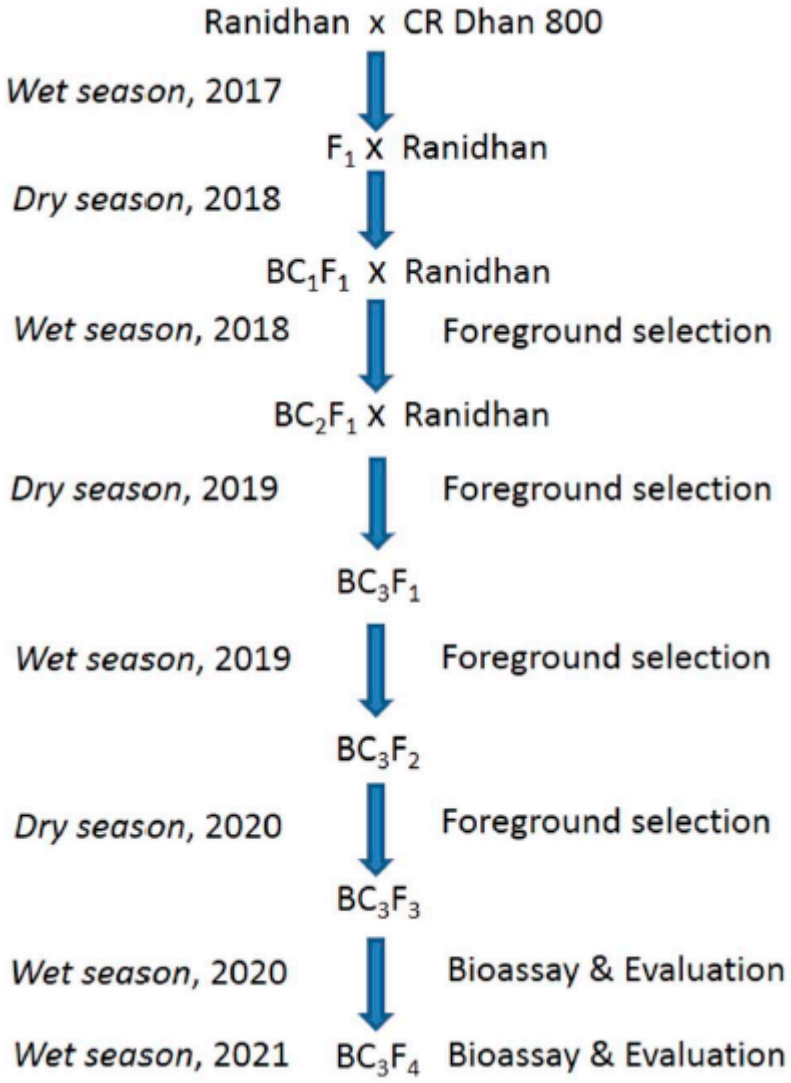


Figure 1. Marker-assisted breeding scheme for transfer of BB resistance genes into the popular variety, Ranidhan.

Table 1. Markers used for the three bacterial blight resistance genes for foreground selection in the marker-assisted backcross breeding program.

BB Resistance Gene	Chromosome Number	Marker Used	Primer Sequences Used for the Gene Detection		Expected Band Size (bp)	Marker Type	Reference
			Forward (5′–3′)	Reverse (5′–3′)			
xa5	5	xa5S (Multiplex) xa5SR/R (Multiplex)	GTCTGGAATTTG-CTCGCGTTCG AGCTCGCCATTCA-AGTTCTTGAG	TGGTAAAGTAGATA-CCTTATCAAACTGGA TGACTTGGTTC-TCCAAGGCTT	410 bp, 310 bp, 180 bp	STS	[28,30]
xa13	8	RG136	TCCCAGAAAG-CTACTACAGC	GCAGACTCCA-GTTTGACTTC	530 bp, 490 bp	STS	[31]
Xa21	11	pTA248	AGACGCGGAA-GGGTGGTTCCCGGA	AGACGCGGTAATCG-AAGATGAAA	1000 bp	STS	[17]

2.2. DNA Isolation, PCR and Marker Analysis

The protocol of Dellaporta et al. was used for DNA extraction [32]. PCR was performed following the standard procedure used in an earlier publication [33]. The markers used for the 3 BB resistance genes in the marker-assisted backcross breeding program are presented in Table 1. For the three target genes, four gene-specific and tightly linked markers were used in the foreground selection (Table 1). The PCR products were separated by using

agarose gel electrophoresis. The gel documentation system (SynGene, Cambridge, UK) was used to capture the image of the bands obtained in the electrophoresis. The data analysis and dendrogram construction were done following the earlier publications [34–36].

2.3. Bioassay of Bacterial Blight

All the BC_3F_3 and BC_3F_4 pyramided lines of forty-five-days-old seedlings along with the parents were inoculated with eight isolates of *Xoo* during the wet seasons of 2020 and 2021. The *Xoo* isolates were maintained at ICAR-NRRI, and Cuttack was used for the bioassay study. The bacterial suspension is prepared by suspending the bacterial mass in sterile water to a concentration of approximately 10^9 cells/mL [37]. Five leaves from different five plants of each entry at the maximum tillering stage were taken for inoculation. The disease symptom and the lesion lengths (LL) were measured after 15 days. The mean lesion length was used for scoring and used as resistant (R) for LL $\leq$ 3.0 cm, moderately resistant (MR) for 3.0 cm < LL $\leq$ 6.0 cm and moderately susceptible (MS) for 6.0 cm < LL $\leq$ 9.0 cm or susceptible (S, LL > 9.0 cm) [38].

2.4. Evaluation of the Genotypes for Various Agro-Morphological Characters

Twenty-five-day-old genotypes carrying the BB resistance genes were transplanted along with the parents during the wet seasons, 2020 and 2021 at ICAR-NRRI, Cuttack. The materials were planted at a spacing of 20 $\times$ 15 cm^2 in three replications in a randomized block design providing a plot size of 5.25 m^2 to each entry with 35 plants per row. The data for ten plants/each entry/replication was recorded for agro-morphologic characters namely, plant height (cm), panicle weight (g), panicles per plant, number of grains per panicle, panicle length (cm), grain breadth (mm), grain length (mm) and 1000-grain weight while the data on days to 50% flowering and plot yield were estimated on the whole plot basis. SAS statistical software was used for the data analysis. Principal component analysis (PCA) for distributing the pyramided and parental lines was drawn by the multivariate analysis for the 10 morphologic and quality traits. A scatter plot was generated by plotting two major components: principal component 1 (PC1) and principal component 2 (PC2). The percentage of variance and eigen value were generated by the interaction of a variance–covariance matrix. All the plots and results of PCA were generated as per a standard procedure following the previous publications [39–41].

3. Results

3.1. Development of Pyramided Lines Carrying BB Resistance Genes

3.1.1. Validation of the Target Resistance Genesin the Parental Lines

The presence of the target genes in the parental lines was confirmed before the beginning of the hybridization and selection activities. Three BB resistance genes were confirmed in the parent, CR Dhan 800, whereas these genes were absent in the recipient parent, Ranidhan (Figure 1). Tightly linked molecular markers were used for the validation of the bacterial blight resistance genes in the parental lines (Table 1).

3.1.2. Screening of the BB Resistance Genes in BC_1F_1Progenies

The popular variety, Ranidhan, was hybridized with the donor parent, CR Dhan 800 and 57 F_1 seeds were produced. These seeds were planted in the next season and the true hybridity was confirmed by genotyping the hybrid plants using the BB gene markers. A true hybrid plant (F_1) was backcrossed with the recipient parent, Ranidhan, and 145 BC_1F_1 crossed seeds were produced. The crossed seeds were raised for BC_1F_1 generation, and foreground selection was done in all the plants using the linked markers for the bacterial blight resistance gene (Figure 2).

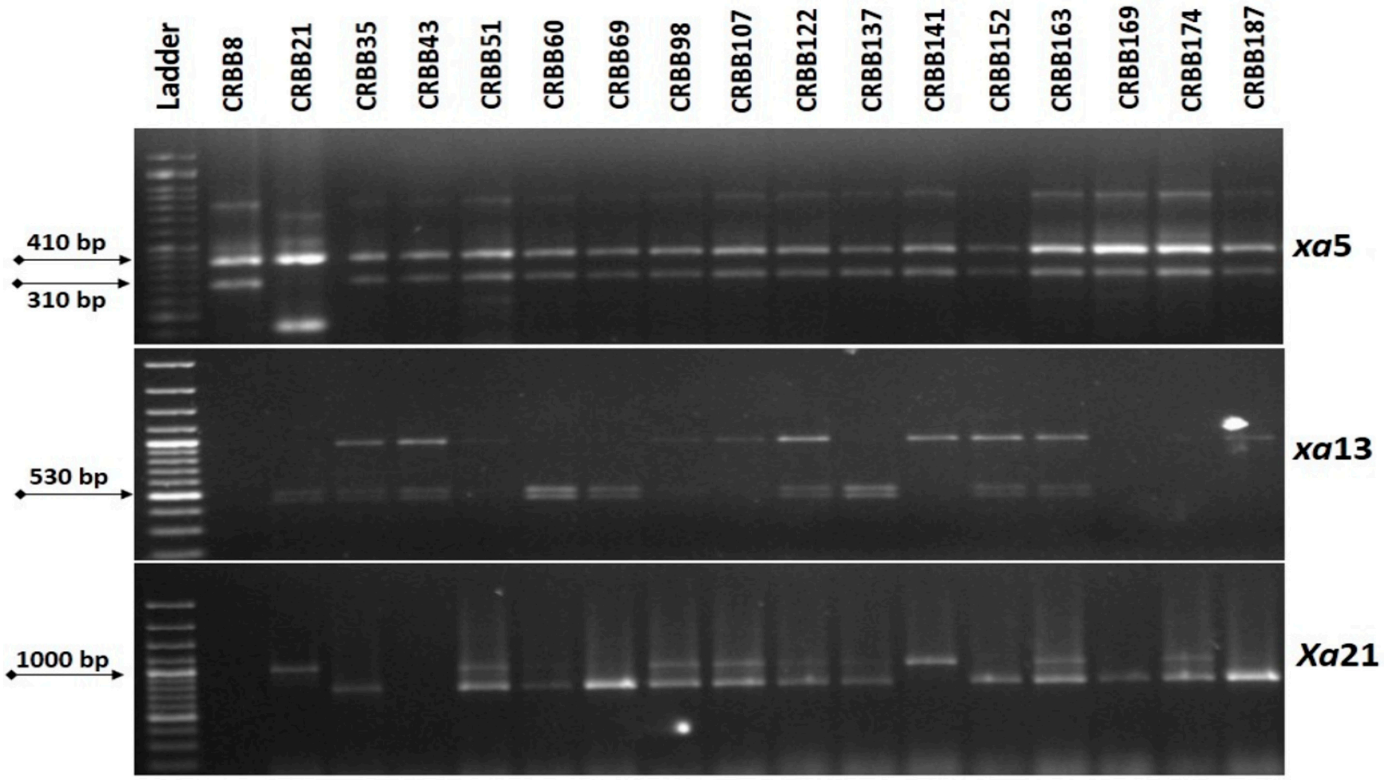


Figure 2. Representative electropherogram generated from the tracking of *Xa21*, *xa13* and *xa5* BB resistance genes in BC_1F_1 progenies of resistance gene pyramiding of Ranidhan.

Out of a total of 145 BC_1F_1 plants, the screening results revealed the presence of the *xa5* gene in 71 derivatives showing an expected band size of 440 bp. Tracking for the presence of the *xa13* gene was confirmed in 35 derivatives with an expected band size of 530 bp. Finally, screening of the 35 positive plants resulted in 17 derivatives with the presence of the *Xa21* gene detected with the expected band size of 530 bp (Figure 2). Out of these 17 plants, the progeny showing more similarity with Ranidhan was selected and backcrossed and 156 seeds were generated.

3.1.3. Screening of the BB Resistance Genes in BC_2F_1 Progenies

All the generated BC_2F_1 seeds were raised in the next season for selection. Foreground selection was performed in all the BC_2F_1 progenies using the closely linked markers with the target BB resistance genes. Out of 156 BC_2F_1 progenies, the screening revealed the presence of the *Xa21* gene in 71 plants. Screening of those 71 plants for the *xa5* gene revealed its presence in 33 plants. Final screening for the *xa5* gene revealed the presence of *Xa21+xa5+xa13* genes in 16 plants (Figure 3). The plant showing more similarity with Ranidhan was backcrossed and 148 seeds were produced.

3.1.4. Marker-Assisted Selection in the BC_3F_1 and BC_3F_2 Progenies

A total of 148 BC_3F_1 seeds were raised in the next season for selection. Target resistance gene selection was performed in all the BC_3F_1 plants using the closely linked markers for the BB resistance genes through foreground screening. The genotyping selection for the target *xa5* gene showed its presence in 67 progenies. Those 67 progenies were genotyped and screened for the *xa13* gene. This analysis identified 32 plants positive for the *xa13* gene and further genotyped for *Xa21*. Finally, 15 plants were positive for the *Xa21* gene (Figure 4). The plant most similar to the Ranidhan plant was self-pollinated.

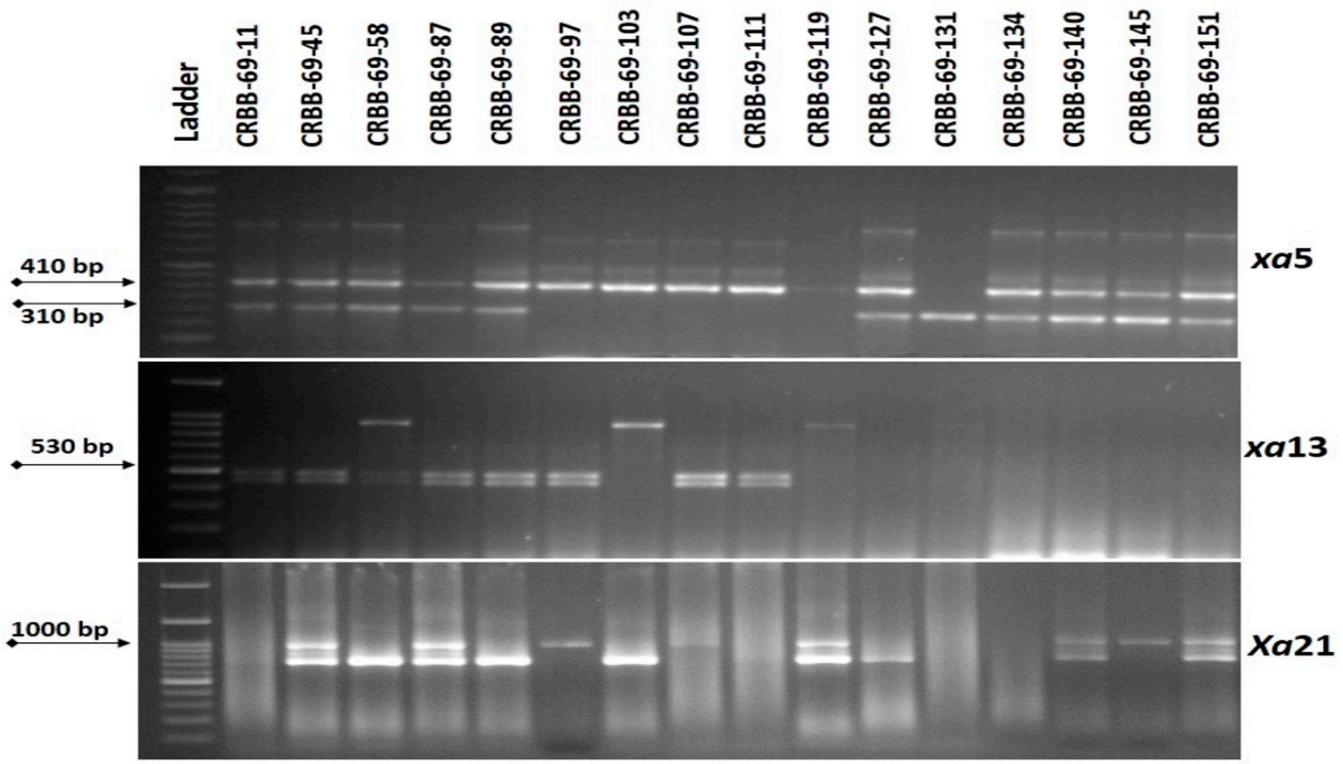


Figure 3. Representative electropherogram generated from the tracking of *Xa21*, *xa13* and *xa5* BB resistance genes in BC_2F_1 progenies of resistance gene pyramiding of Ranidhan.

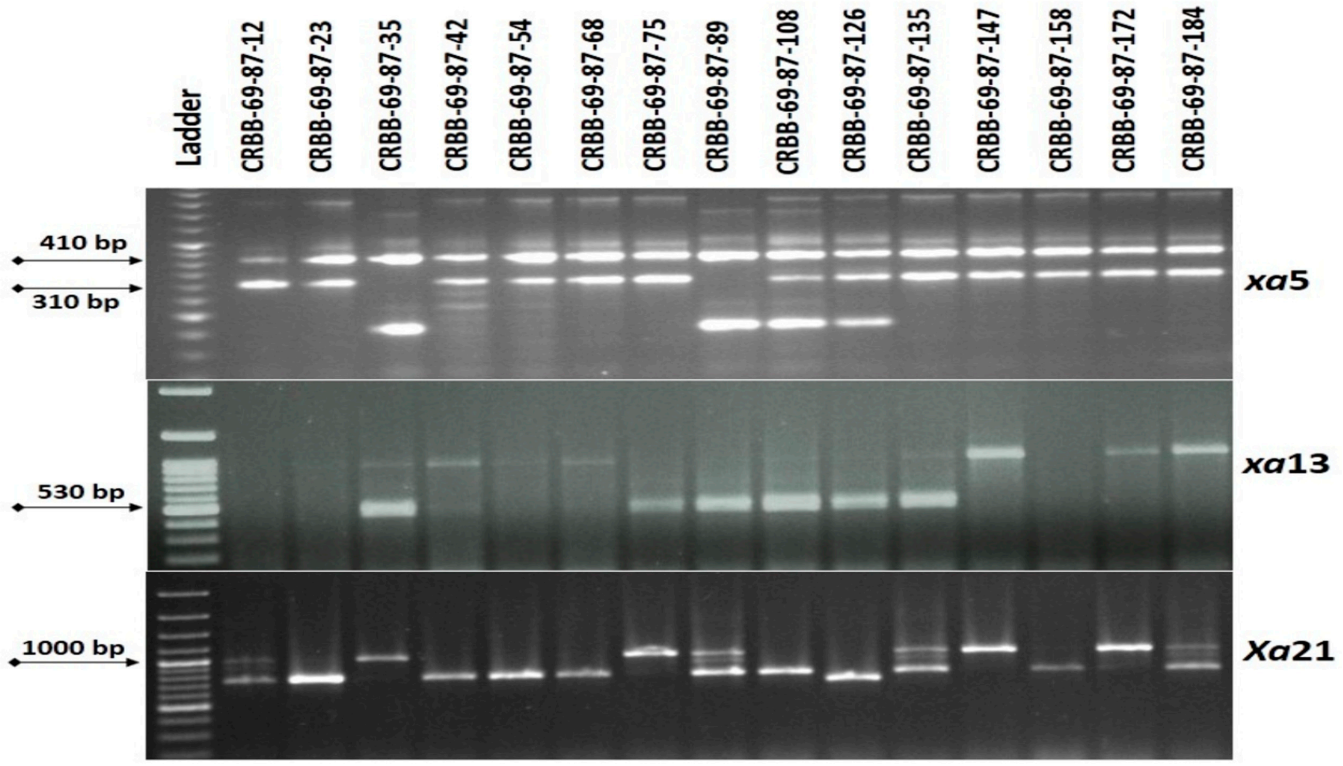


Figure 4. Representative electropherogram generated from the tracking of *Xa21*, *xa13* and *xa5* BB resistance genes in B_3F_1 progenies of resistance gene pyramiding of Ranidhan.

The self pollinated seeds of the previous generation were raised during the next season and 225 BC_3F_2 generation progenies were tracked via foreground selection using the BB

resistance gene markers. The genotyping screening of all the 225 BC_3F_2 progenies showed the presence of *Xa21* gene in 53 plants. These 53 positive plants were checked for *xa5* gene and found its presence in 13 plants. Out of the thirteen plants, finally three progenies carrying *xa5+Xa21* resistance genes, two plants containing *Xa21+xa13* genes and two plants containing *xa5+xa13 genes* were identified to be homozygous (Figure 5).

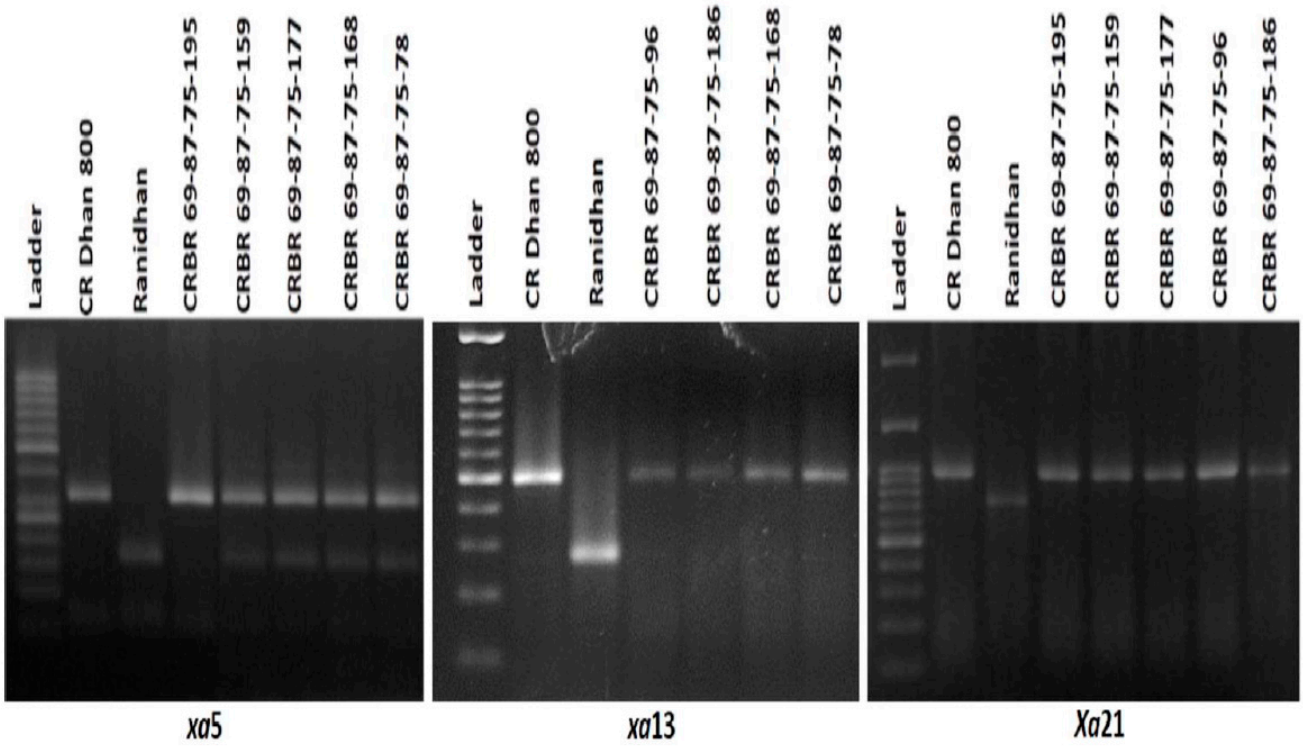


Figure 5. Representative electropherogram generated from the tracking of *Xa21*, *xa13* and *xa5* BB resistance genes in BC_3F_2 progenies of resistance gene pyramiding of Ranidhan.

3.2. Bioassay of the Pyramided Lines against Bacterial Blight Pathogens

Twelve test entries including seven BC_3F_4 generation pyramided lines (three plants carrying *xa5+Xa21* genes, two plants with *Xa21+xa13* genes and two plants containing *xa5+xa13* genes) along with one plant each carrying *xa5/xa13/Xa21* genes and donor parent CR Dhan 800 and recipient parent Ranidhan were screened after 15 days of artificial inoculation using eight *Xoo* isolates. The bioassay results confirmed the resistance and susceptible reaction of the donor and the recurrent parents, respectively, based on the phenotyping results of BB disease reaction during wet seasons 2020 and 2021. The donor parent, CR Dhan 800, showed low average lesion lengths (2.2–2.6 cm) while the recurrent parent, Ranidhan, had longer lesion lengths (9.5–11.1 cm) (Table 2). The results based on the phenotypic data showed that the developed pyramided lines were better than the recurrent parent for disease resistance against the BB isolates. Gene combinations of *xa5+Xa21* showed a range of 3.9–4.3 cm and *xa13+Xa21* showed a range of 4.3–5.3 while *xa5+xa13* gene combinations showed a range of 6.1–6.5 cm. The pyramided plants carrying *xa13+Xa21* and *xa5+Xa21* gene combinations showed higher levels of BB disease resistance with shorter lesion lengths against all the BB isolates. However, all the plants tested carrying different resistance gene combinations did not show any susceptible reaction to any of the eight BB strains tested. From this investigation, it may be inferred that that the of severity of the disease may be in the order of *Xa21+xa5* < *Xa21+xa13* < *xa13+xa5* resistance genes combinations in conferring BB resistance.

Table 2. BB disease reaction of parental, BC_3F_3, and BC_3F_4 pyramided lines against different *Xoo* strains.

Sl. No.	Pyramided Lines/Xoo Strains	Gene Combination	Mean Lesion Length in cm (Mean ± Standard Error)								
			Xa-17	**Xa-7**	**xa-2**	**xb-7**	**xc-4**	**xd-1**	**xa-1**	**xa-5**	**Disease Reaction**
1	CRBR 69-87-75-195	*Xa21+xa5*	4.1 ± 0.54	4.2 ± 0.57	4.3 ± 0.52	4.2 ± 0.70	4.3 ± 0.60	4.1 ± 0.56	4.0 ± 0.80	4.2 ± 0.53	MR
2	CRBR 69-87-75-159	*Xa21+xa5*	4.2 ± 0.64	3.9 ± 0.81	3.9 ± 1.0	4.1 ± 0.65	4.3 ± 0.60	4.2 ± 0.70	4.3 ± 053	4.1 ± 0.65	MR
3	CRBR 69-87-75-177	*Xa21+xa5*	4.3 ± 0.56	4.2 ± 0.64	4.1 ± 0.75	4.2 ± 0.41	4.3 ± 0.55	4.2 ± 0.42	4.1 ± 0.59	4.2 ± 0.65	MR
4	CRBR 69-87-75-96	*Xa21+xa13*	5.2 ± 0.53	5.2 ± 0.45	5.3 ± 0.50	5.4 ± 0.40	4.9 ± 0.64	4.3 ± 0.45	4.9 ± 0.90	5.0 ± 0.80	MR
5	CRBR 69-87-75-186	*Xa21+xa13*	4.9 ± 0.72	5.1 ± 0.52	5.2 ± 0.51	5.2 ± 0.54	5.1 ± 0.63	5.3 ± 0.50	5.3 ± 0.38	4.9 ± 0.78	MR
6	CRBR 69-87-75-168	*xa5+xa13*	6.3 ± 0.58	6.2 ± 063	6.1±0.80	6.5 ± 0.40	6.3 ± 0.59	6.3 ± 0.60	6.2 ± 0.65	6.3 ± 0.57	MS
7	CRBR 69-87-75-78	*xa5+xa13*	6.2 ± 0.65	6.4 ± 0.42	6.3 ± 0.52	6.4 ± 0.47	6.1 ± 0.57	6.2 ± 0.68	6.3 ± 0.57	6.3 ± 0.56	MS
8	CRBR 69-87-75-114	*xa5*	7.7 ± 0.45	7.8 ± 0.48	7.7 ± 0.55	7.9 ± 0.43	7.7 ± 0.53	7.8 ± 0.51	7.9 ± 0.49	7.7 ± 0.43	MS
9	CRBR 69-87-75-51	*xa13*	8.1 ± 0.53	8.3 ± 0.46	8.4 ± 0.43	8.2 ± 0.58	8.1 ± 0.62	8.0 ± 0.61	8.4 ± 0.47	8.3 ± 0.55	MS
10	CRBR 69-87-75-132	*Xa21*	7.2 ± 0.56	7.4 ± 0.38	7.3 ± 0.63	7.5 ± 0.54	7.2 ± 0.62	7.1 ± 0.52	7.2 ± 0.58	7.3 ± 0.56	MS
11	CR Dhan 800	*xa5+xa13+Xa21*	2.6 ± 0.35	2.5 ± 0.45	2.4 ± 0.42	2.2 ± 0.48	2.2 ± 0.52	2.4 ± 0.44	2.5 ± 0.38	2.2 ± 0.6	R
12	Ranidhan	-	10.6 ± 1.2	10.4 ± 1.4	10.8 ± 1.1	9.5 ± 2.4	11.1 ± 0.8	9.8 ± 1.6	10.2 ± 1.7	10.6 ± 1.3	S

3.3. Evaluation of the Pyramided Lines for Yield, Agro-Morphologic and Grain Quality Traits

Twelve genotypes including the sevenpyramided lines carrying two BB resistance genes and three lines containing one resistance gene at BC_3F_4 generations along with the parents were evaluated during wet seasons, 2020 and 2021 at ICAR-NRRI, Cuttack. Ten traits for yield, agro-morphologic and grain quality traits were recorded. The popular variety, Ranidhan, produced a mean grain yield of 5.82 t/ha. All test entries carrying two BB resistance target genes showed grain yield higher than the recipient parent, Ranidhan (Table 3). The majority of the two resistance genes containing pyramided lines were similar to the recipient parent, Ranidhan, for the main agro-morphologic and quality traits (Figure 6).

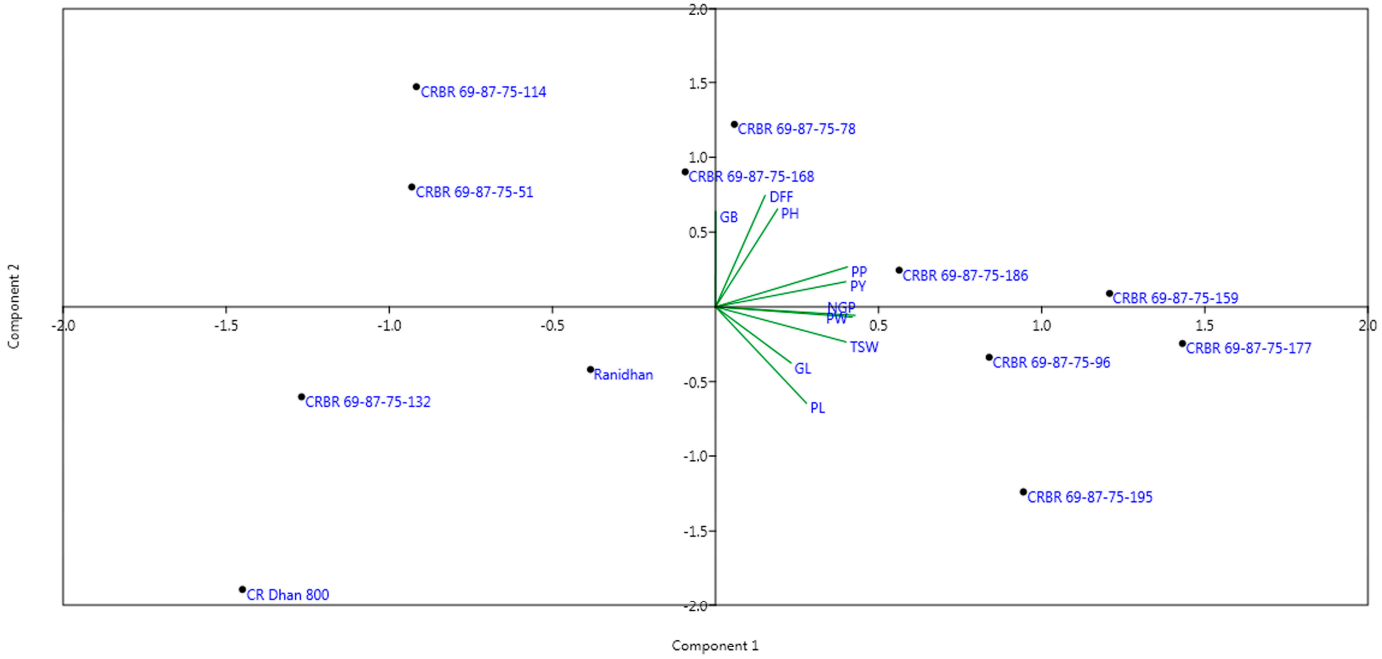


Figure 6. Biplot diagram generated using the 10 traits for yield, agro-morpholic and grain quality characters of the pyramided and parental lines.

The pyramided line CRBR 69-87-75-177 showed the highest grain yield 67.1 q/ha followed by CRBR 69-87-75-159 (63.8 q/ha) and CRBR 69-87-75-96 (62.7 q/ha) (Table 3). A total of six pyramided lines showed more yield than the recipient parent. The best-pyramided line showed a higher yield of 15.29% than the recipient variety, Ranidhan. The best-pyramided line showed a 5.52 cm grain length, while the recipient parent had 5.62 cm. The popular variety and the best-pyramided line had the same grain breadth of 2.32 cm. Seven pyramided lines were observed to be at par with many of the agro-morphologic traits.

Table 3. Grain yield, agro-morphologic and grain quality traits of parental and selected BC_3F_3 and BC_3F_4 pyramided lines.

Serial Number	Pyramided Lines	Plant Height (cm)	Days to 50% Flowering	Panicles/Plant	Panicle Length (cm)	No of Grains/ Panicle	1000- Seed Weight(g)	Panicle Weight (g)	Grain Length (mm)	Grain Breadth (mm)	Plot Yield (q/ha)
1	CRBR 69-87-75-195	104.2	114	16	29.3	150.0	21.5	3.2	5.76	2.29	62.1
2	CRBR 69-87-75-159	106.2	115	17	27.7	153.3	21.7	3.3	5.68	2.34	63.8
3	CRBR 69-87-75-177	105.5	113	18	28.7	157.0	21.9	3.3	5.52	2.32	67.1
4	CRBR 69-87-75-96	109.2	112	16	27.3	153.3	21.3	3.2	5.59	2.30	63.7
5	CRBR 69-87-75-186	108.2	116	17	25.7	145.0	21.0	3.1	5.53	2.29	61.6
6	CRBR 69-87-75-168	107.2	115	15	22.7	140.0	20.4	3.1	5.57	2.34	59.8
7	CRBR 69-87-75-78	108.5	117	16	23.3	143.7	20.6	2.9	5.49	2.33	60.3
8	CRBR 69-87-75-114	107.2	116	15	22.7	128.3	19.4	2.6	5.36	2.34	59.5
9	CRBR 69-87-75-51	107.2	115	14	23.7	125.0	19.2	2.5	5.57	2.33	59.9
10	CRBR 69-87-75-132	102.5	112	13	25.3	130.0	19.8	2.5	5.36	2.29	57.5
11	CR Dhan 800	100.5	106	12	26.3	125.0	20.1	2.6	5.57	2.30	59.6
12	Ranidhan	101.5	113	15	25.5	137.7	19.6	2.9	5.62	2.32	58.2
	$CD_{5\%}$	9.34	3.20	0.96	3.25	20.34	2.67	0.47	0.26	0.08	10.6
	CV%	5.15	1.64	3.64	7.38	8.43	7.58	9.35	2.77	2.12	10.17

4. Discussion

The integration of molecular markers for the selection of desired plants in a backcross breeding program not only increases the precision in transferring the target genes into the recipient variety but also reduces the duration of the selection cycle compared to the classical breeding approach. The selection of progenies in three back cross generations followed by two successive selfing generations was sufficient for the transfer of the two gene combinations into the susceptible variety. Ranidhan is a popular variety in late maturing rice varieties but is highly susceptible to the BB disease. Under lowland rainfed ecology, controlling the BB disease is a relatively hard and difficult task. Developing BB resistant lines in the background of the popular high-yielding variety, Ranidhan, took less time and was easier by using fewer generations through the integration of molecular markers in the breeding program. The pyramided resistance genes in the developed lines showed a higher level of tolerance against the virulent BB isolates. The development and release of BB pyramided lines by employing marker-assisted backcross breeding has been successful, as reported in earlier publications. It illustrated less time, more precision and more eco-friendliness [1,15,24–27,29,41–44].

Two resistance genes carrying pyramided lines conferred higher BB resistance compared to the introgressed line carrying a single resistance gene. Results indicated that the two gene combinations (*Xa21+xa5)* pyramided lines showed relatively smaller lesion lengths followed by *Xa21+xa13* gene combinations pyramided lines compared to the recipient variety, Ranidhan. The pyramided lines carrying the dominant resistance gene, *Xa21* along with *xa5* or *xa13* showed higher resistance than single gene carrying lines. Similar findings were also reported by Sanchez et al. [19]; Singh et al. [17] and Pradhan et al. [45] for conferring higher resistance using the *Xa21* gene in rice. The synergistic action of the resistance genes or quantitative complementation between the BB resistance genes might result in higher resistance in rice [19,45].

Twelve genotypes including seven BC_3F_4 pyramided lines (three plants carrying *xa5+Xa21* genes, two plants containing *xa13+Xa21* genes and two plants containing *xa5+xa13* genes) along with one plant each of carrying *xa5/xa13/Xa21* genes and donor parent, CR Dhan 800 and recipient parent, Ranidhan, were screened using eight *Xoo* isolates. Validation confirmed the resistance and susceptible reaction of the donor and the recurrent parents, respectively, through phenotyping the results of BB disease reaction. The BB donor parent showed lower average lesion lengths (2.2–2.6 cm) while the popular variety, Ranidhan, had higher lesion lengths (9.5–11.1 cm) (Table 2). The bioassay result confirmed that the pyramided lines were better in resisting BB disease compared to the recurrent parent. Gene combinations of *xa5+Xa21* showed a range of 3.9–5.3 cm and *xa13+Xa21* showed a range of 4.3–5.3, while *xa5+xa13* gene combinations showed a range of 6.1–6.5 cm. Results of the bioassay study indicated that the pyramided lines carrying target gene combinations did not show a susceptible reaction to any of the eight isolates employed. The gene pyramids in *Xa21+xa5* and *Xa21+xa13* resistance gene combinations showed better BB disease resistance showing smaller mean lesion lengths against all BB pathotypes. From this investigation, it is concluded that gene combinations conferred resistance in the order of *Xa21+xa5* < *Xa21+xa13* < *xa13+xa5.*

The combined evaluation results of the two seasons revealed a higher grain yield of the pyramided lines than the recipient popular variety, Ranidhan (Table 3). The majority of the pyramided lines carrying resistance genes were similar to the recipient parent, Ranidhan, for important agro-morphologic and grain quality traits (Figure 6). The pyramided line CRBR 69-87-75-177 showed the highest grain yield 67.1qt/ha followed by CRBR 69-87-75-159 (63.8 q/ha) and CRBR 69-87-75-96 (62.7 q/ha) (Table 3). A total of six pyramided lines carrying resistance genes were higher yielders than the recipient parent. The best-pyramided line showed a yield advantage of 15.3% over the recurrent parent. The best-pyramided line showed a 5.52 cm grain length, while the recipient parent had 5.62 cm. The best-pyramided line and the recipient parent had the same grain breadth of 2.32 cm. Seven pyramided lines were observed to be at par with many of the agro-morphologic traits.

Integration of molecular markers in classical breeding program for the selection of the BB resistance in the pyramids conferred a higher level of resistance to the BB disease. Single resistance gene incorporation is broken down by the rich diversity of the genetically distinct virulent *Xoo* strains available in the different agro-climatic zones of the country. Development of the pyramided lines carrying two BB resistance genes in suitable combination compared to the single resistance gene carrying will provide alternative variety to the Ranidhan growers. These pyramided lines containing *xa5+Xa21* and *Xa21+xa13* can also be useful for the other BB endemic regions of the country. The investigation clearly indicates that the deployment of a suitable resistance gene combination through gene pyramiding will enhance the level of resistance against this disease.

5. Conclusions

Marker-assisted breeding is a precise breeding approach for pyramiding BB resistance genes in rice through backcross breeding. It was successful in developing superior BB resistant lines, which conferred a higher level of resistance in the background of a popular variety, Ranidhan. The developed pyramided lines can either be useful as promising donors for BB resistance or tested under multi-location testing for release as a variety in the state/country. BB resistant pyramided lines developed through marker assisted backcrossing will provide a solution in the BB endemic areas of the region/country. These pyramided lines are expected to give a high impact not only on yield stability but also the sustainability of the rainfed ecology, particularly ineastern India where the majority of rice areas are in rainfed lowlands.

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