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Research Article

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Evaluation of Pyramided Rice Genotypes Derived from Cross Between CSR-30 and IRBB-60 Basmati Variety against Bacterial Leaf Blight

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Abstract

Bacterial leaf blight (BB) disease caused by *Xanthomonas oryzae pv. oryzae* is one of the most severe diseases effecting Basmati rice production in India. CSR-30 is widely grown in Haryana and is extremely popular amongst rice farmers and consumers because of its salt tolerance, aroma, high yield, medium slender grains and excellent cooking and eating qualities. CSR-30 lacks tolerance to BB. The present investigation was therefore planned to introgress BB resistance genes (*xa5, xa13* & *Xa21*) from IRBB-60 to CSR-30. The pyramided BC_3F_1 genotypes were evaluated for BB incidence under artificial field inoculation. Scoring of inoculated plant was done 14 days after inoculation. The triple and double resistance genes pyramided genotypes provided enhanced resistance as expressed by smaller mean lesion length in comparison to genotypes with individual genes.

Keywords

Basmati rice; Bacterial leaf blight; Gene pyramiding; Resistance genes; *Xanthomonas oryzae* pv. *oryzae*

Introduction

BB is one of the most devastating diseases of rice worldwide [1,2]. It affects rate of photosynthesis and lead to yield losses of up to 80-100% in severe cases [3,4]. The main symptoms of the disease are water soaked stripes along the margin of leaf blades, which enlarges later on and turn yellow. These lesions may cover the entire blade, may extend to the lower end of leaf sheath. Several rice resistance genes are expressed at the highest level only in the adult stage [5,6]. *Xa21* mediated resistance gene was shown to be expressed since the seedling stage but the plants were found susceptible, and *Xa7* gene showed broad resistance only in adult plants [7]. On the contrary, *xa5* gene could confer resistance at all growth stages and exhibit a broad spectrum of resistance to *Xoo* isolates (India and Nepal) [8].

The development of resistant cultivars is the best approach of protecting the rice from BB disease [9]. Gene to gene interaction of BB with rice makes it an effective model to study plant pathogen interaction [10]. Long-term cultivation of rice varieties carrying single resistance gene has resulted in a significant shift in pathogen-race frequency and consequent breakdown of resistance [11]. Pyramiding of multiple resistance genes in the background of modern high yielding varieties is a tangible solution to resistance breakdown. The probability of simultaneous pathogen mutations for virulence to defeat two or more effective genes is much lower than with a single gene [12]. In view of the importance of genetic resistance for disease control, studies were undertaken to evaluate the pyramided rice genotypes against BB disease.

Materials and Methods

The experimental material for the present study consisted of BB resistance genes pyramided BC_3F_1 genotypes derived from cross between BB susceptible Basmati rice variety CSR-30 (recurrent parent) and donor BB resistant IRBB-60 (having genes, *xa5*, *xa13* and *Xa21*). The pyramided BC_3F_1 genotypes were evaluated for disease reaction and compared with the donor parent, IRBB-60.

Isolation of bacteria (*Xanthomonas oryzae* pv. *oryzae*)

Infected rice leaves showing BB symptoms were collected from the BB infected fields of RRS, Kaul. These leaves were surface-sterilized with 2% sodium hypochlorite for 1 min and washed twice with sterile distilled water. The leaves were then cut into 0.5 cm pieces and placed in 10 ml of sterile distilled water. The cells were allowed to ooze from leaves into sterile water and streaked for single-colony isolation on PSA plates. The *Xoo* isolate was multiplied and maintained on peptone sucrose agar (PSA) at 28°C. These isolates were preserved in glycerol at -70°C (Table 1).

Artificial inoculation

Plants selected on the basis of molecular marker analysis from the BC_3F_1 generation (CSR-30 x IRBB-60) carrying resistance genes (*Xa21*, *xa13* and *xa5*) individually and in combinations, along with the control, were inoculated with the predominant *Xoo* isolate prevalent in Haryana State using a bacterial suspension of 10^9cells/ml [13]. The plants were clip inoculated at maximum tillering stage. The leaf blades were inoculated by clipping with scissors at 3 cm below the leaf tips. On an average five leaves per plant were inoculated and the disease incidence (DI) using 0-5 scale (Table 2) was measured 14 days after inoculation.

Results

The *Xa21*, *xa13* and *xa5* pyramided lines in different combinations (Table 3) were evaluated for their resistance to BB in field and net house

Table 1: Composition of Peptone Sucrose Agar (PSA) media.

Composition of Peptone Sucrose Agar (PSA) media	
Sucrose	5.0g
Sodium glutamate	1.0g
Ferrous sulphate	0.25g
Yeast extract	2.5g
Peptone	10.0g
Agar	15.0g
pH	6.0

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using the *Xanthomonas. oryzae* strain isolated from the BB infected fields of RRS, Kaul, CCSHAU, Hisar. Isolation of bacteria was done by adapting streak plate method. On PSA plates, *Xanthomonas oryzae* pv. *oryzae* having circular, entire, smooth, convex, opaque, whitish yellow at first and straw yellow later was identified. Well separated colonies of the isolate were picked up and streaked on PSA media and incubated at 28°C for 72 hours. The pure colonies obtained were again streaked on PSA slants and kept for incubation at 28°C for 72 hours. The cultures so obtained were stored in the refrigerator at 5°C, which served as a stock culture for further studies. The pyramided lines along with the control were inoculated using a bacterial suspension of 10^9cells/ml. The leaf blades were inoculated by clipping with scissors at 3 cm below the leaf tips. On an average five leaves per plant were inoculated and the disease incidence using 0-5 scale was measured 14 days after inoculation.

The three-gene pyramided BC_3F_1 plants derived in this study from the cross CSR-30 x IRBB-60, were found to be equally effective against the virulent *Xoo* strain (mean lesion length of 0.4 cm) as compared to the donor line IRBB-60 (mean lesion length of 0.5 cm). Also the pyramided lines having either *Xa21* (mean lesion length of 1.2 cm) or *xa5* (mean lesion length of 1.1 cm) resistance genes alone were found to be resistant or moderately resistant to the BB disease. However, pyramided lines with *xa13* gene (mean lesion length of 4.8 cm) alone were found to be susceptible to BLB disease. The pyramided lines (two gene or three gene combinations) had a higher level of resistance and broader spectrum of resistance than parental lines or lines with a single gene. The *Xa21* and *xa5* pyramided lines exhibited a mean lesion length of 0.8 cm whereas those having *Xa21* and *xa13* showed a mean lesion length of 1.5 cm. The pyramided lines having both *xa13* and *xa5* showed a mean lesion length of 2.8 cm (Table 4). This might be due to interaction and/or complementation between the resistance genes (Figure 1).

Discussion

As compared to the recurrent parent CSR-30, the pyramided lines with two to three gene (*Xa21, xa13* and *xa5*) combinations exhibited high level of resistance to BB disease. The lines containing *Xa21*or *xa5* alone exhibited moderate BB resistance. However, lines with *xa-13* gene alone were found to be susceptible to BB disease. The results indicated that the genes in combinations were more effective against the pathogen than a single gene (Figure 2).

Based on the above results, we infer that, individually, *xa5* and *Xa21* were more effective resistance genes than *xa13*. Li et al. [14] reported that a high level of durable resistance to *Xoo* can be achieved by the cumulative effects of multiple QTLs, including the residual effects of defeated major resistance genes. They revealed a complex genetic network of epistatic effects between resistance genes and QTLs for resistance in rice. They reported that resistance to specific *Xoo* strains is governed by both major resistance genes with a qualitative effect that condition complete resistance and polygenes with a quantitative effect (QTL) that condition partial resistance. In a similar study, Singh et al. [15] reported that the genes in combinations were more effective against the pathogen than a single gene, by inoculating the selected BC_2F_3 PR106 lines homozygous for each of the individual genes and with different combinations with the 17 isolates of *Xoo* prevalent in Punjab. They found that *Xa21* was the most effective gene, followed by *xa5* and that gene *xa13* was the least effective. These results were in accordance with those reported in our study. Huang et al. (2012), in another study, introgressed four bacterial leaf blight (BB) resistance genes, *Xa7, Xa21, Xa22* and *Xa23*, into an elite hybrid rice restorer line Huahui 1035, using MAS and found that restorer lines (HBQ809 and HBQ810) with *Xa23* gene were resistant to all eleven Chinese representative *Xoo* races, showing broad spectrum resistance to BB. Dokku et al. [16] through marker assisted backcrossing transferred three BB resistance genes *i.e. xa5*, *xa13* and *Xa21* from IRBB60 to Tapaswini having *Xa4* gene. The four gene combination expressed higher levels of resistance in comparison to all other gene combinations and genes in combination were more effective than a single gene. *Xa21* was most effective with shorter lesions lengths followed by *xa13* while lines with *xa5* were susceptible to all isolates except *xd-1*. The findings of this work is contrary to that concluded by our study where *Xa21* was found most effective against BB followed by *xa5* and *xa13* gene.

Table 2: Disease rating using 0-5 scale.

Infection (%)	Score	Host response
0	0	Highly resistant (HR)
1-10	1	Resistant (R)
10-30	2	Moderately resistant (MR)
30-50	3	Moderately susceptible (MS)
50-75	4	Susceptible (S)
75-100	5	Highly susceptible (HS)

Table 3: Number of BC_3F_1 plants with multiple resistance gene combinations.

S. No	Gene combinations	(CSR-30 x IRBB 60) BC_3F_1 plants	Line numbers
1	*Xa21/Xa21Xa13/Xa13 Xa5/Xa5*	17	R-1, R-2, R-7, R-9, R-11, R-12, R-21, R-23, R-29, R-36, R-37, R-49, R-52, R-66, R-78, R-92, R-101
2	*xa21/xa21xa13/xa13 Xa5/Xa5*	14	R-3, R-5, R-10, R-13, R-17, R-24, R-31, R-35, R-62, R-72, R-86,R-97, R-102, R-108
3	*xa21/xa21Xa13/Xa13 xa5/xa5*	8	R-22, R-47, R-76, R-82, R-98, R-105, R-106, R-112,
4	*Xa21/Xa21xa13/xa13 Xa5/Xa5*	36	R-4, R-6, R-8, R-15, R-18, R-19, R-20, R-25, R-27, R-30, R-33, R-34, R-45, R-46, R-50, R-54, R-57, R- R-60, R-61, R-65, R-67, R-71, R-73, R-75, R-77, R-79, R-80, R-85, R-88, R-91, R-96, R-99, R-100,R-103, R-107, R-110
5	*Xa21/Xa21Xa13/Xa13 xa5/xa5*	5	R-14, R-39, R-43, R-44, R-93
6	*xa21/xa21xa13/xa13 xa5/xa5*	3	R-26, R-70, R-87
7	*Xa21/Xa21xa13/xa13 xa5/xa5*	15	R-28, R-32, R-38, R-42, R-58, R-59, R-63, R-68, R-69, R-74, R-81, R-90, R-94, R-95, R-111

Table 4: Disease reaction of BC_3F_1 rice genotypes (containing one to three BB resistance genes) to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Five point rating scale for scoring/screening of BB disease).

S. No	Parents and pyramid lines	*Xa21*	*xa13*	*xa5*	Disease rating	Reaction category	BB mean lesion length (cm)
1	IRBB-60	+	+	+	1	HR	0.5
2	CSR-30	-	-	-	5	HS	18.8
3	R-1	+	-	-	2	MR	0.9
4	R-2	+	-	-	2	MR	1.6
5	R-3	-	+	-	3	MS	3.7
6	R-4**	+	+	-	2	MR	1.4
7	R-5	-	+	-	4	S	4.2
8	R-6**	+	+	-	2	MR	1.6
9	R-7	+	-	-	2	MR	0.7
10	R-8**	+	+	-	2	MR	1.5
11	R-9	+	-	-	2	MR	0.8
12	R-10	-	+	-	4	S	5.1
13	R-11	+	-	-	3	MS	3.1
14	R-12	+	-	-	2	MR	1.6
15	R-13	-	+	-	4	S	5.7
16	R-14**	+	-	+	2	MR	1.0
17	R-15**	+	+	-	2	MR	1.6
18	R-17	-	+	-	3	MS	4.4
19	R-18**	+	+	-	2	MR	1.4
20	R-19**	+	+	-	2	MR	1.5
21	R-20**	+	+	-	2	MR	1.6
22	R-21	+	-	-	2	MR	0.6
23	R-22	-	-	+	2	MR	0.9
24	R-23	+	-	-	2	MR	0.4
25	R-24	-	+	-	3	MS	4.7
26	R-25**	+	+	-	2	MR	1.5
27	R-26**	-	+	+	2	MR	1.9
28	R-27**	+	+	-	0	MR	1.1
29	R-28*	+	+	+	0	HR	0.3
30	R-29	+	-	-	2	MR	1.2
31	R-30**	+	+	-	2	MR	1.1
32	R-31	-	+	-	4	S	4.3
33	R-32*	+	+	+	0	HR	0.3
34	R-33**	+	+	-	2	MR	1.6
35	R-34**	+	+	-	2	MR	1.5
36	R-35	-	+	-	3	MS	4.2
37	R-36	+	-	-	2	MR	1.9
38	R-37	+	-	-	2	MR	1.7
39	R-38*	+	+	+	1	R	0.2
40	R-39**	+	-	+	3	MS	0.8
41	R-42*	+	+	+	0	HR	0.4
42	R-43**	+	-	+	1	R	0.6
43	R-44	+	-	+	1	R	0.8
44	R-45**	+	+	-	2	MR	1.7
45	R-46**	+	+	-	2	MR	1.4
46	R-47	-	-	+	1	R	1.6
47	R-49	+	-	-	2	MR	0.5
48	R-50**	+	+	-	1	R	1.0
49	R-52	+	-	-	2	MR	0.4
50	R-54**	+	+	-	2	MR	1.9

51	R-57**	+	+	-	2	MR	1.7
52	R-58*	+	+	+	1	R	0.4
53	R-59*	+	+	+	0	HR	0.2
54	R-60**	+	+	-	2	MR	1.2
55	R-61**	+	+	-	2	MR	1.7
56	R-62	-	+	-	4	S	5.5
57	R-63*	+	+	+	0	HR	0.3
58	R-65**	+	+	-	2	MR	1.5
59	R-66	+	-	-	2	MR	1.9
60	R-67**	+	+	-	1	R	1.0
61	R-68*	+	+	+	0	HR	0.3
62	R-69*	+	+	+	1	R	0.4
63	R-70**	-	+	+	2	MR	3.4
64	R-71**	+	+	-	2	MR	1.5
65	R-72	-	+	-	4	S	5.5
66	R-73**	+	+	-	2	MR	1.2
67	R-74*	+	+	+	2	MR	0.2
68	R-75**	+	+	-	2	MR	1.8
69	R-76	-	-	+	2	MR	0.9
70	R-77**	+	+	-	2	MR	1.9
71	R-78	+	-	-	2	MR	0.6
72	R-79**	+	+	-	2	MR	1.5
73	R-80**	+	+	-	2	MR	1.1
74	R-81*	+	+	+	0	HR	0.3
75	R-82	-	-	+	2	MR	1.4
76	R-85**	+	+	-	2	MR	1.7
77	R-86	-	+	-	4	S	5.1
78	R-87**	-	+	+	2	MR	2.9
79	R-88**	+	+	-	2	MR	1.4
80	R-90*	+	+	+	0	HR	0.3
81	R-91**	+	+	-	2	MR	1.5
82	R-92	+	-	-	2	MR	0.7
83	R-93**	+	-	+	1	R	0.5
84	R-94*	+	+	+	0	HR	0.3
85	R-95*	+	+	+	1	R	0.4
86	R-96**	+	+	-	2	MR	1.3
87	R-97	-	+	-	3	MS	4.1
88	R-98	-	-	+	2	MR	0.8
89	R-99**	+	+	-	2	MR	1.6
90	R-100**	+	+	-	2	MR	1.9
91	R-101	+	-	-	2	MR	1.3
92	R-102	-	+	-	3	MS	5.1
93	R-103**	+	+	-	2	MR	1.3
94	R-105	-	-	+	2	MR	0.9
95	R-106	-	-	+	2	MR	1.0
96	R-107**	+	+	-	2	MR	2.0
97	R-108	-	+	-	4	S	4.8
98	R-110**	+	+	-	2	MR	1.4
99	R-111*	+	+	+	0	HR	0.4
100	R-112	-	-	+	1	R	0.7

Note: *indicates three-gene pyramided genotypes

**indicates two-gene pyramided genotypes

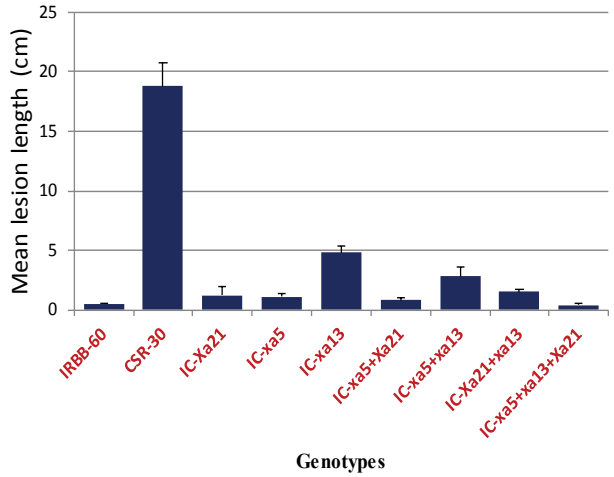

Figure 1: Disease reaction of the donor parent (IRBB-60), recurrent parent (CSR-30) and pyramided BC_3F_1 genotypes (carrying one to three BB resistance genes).

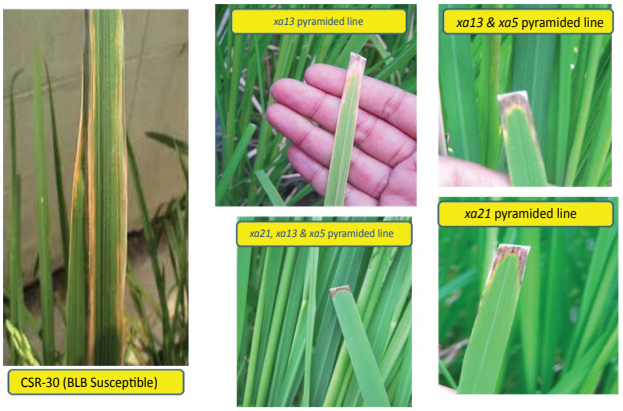

Figure 2: Disease scoring after fourteen days of inoculation in the lines carrying multiple gene combinations (one to three BB resistance gene combinations).

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