Int.J.Curr.Microbiol.App.Sci (2019) *8*(9): 2755-2765

International Journal of Current Microbiology and Applied Sciences
***ISSN: 2319-7706* Volume 8 Number 09 (2019)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2019.809.317

Evaluation of Resistance of Rice Genotypes (Derived from the Cross between HKR-47 and IRBB-60) against Bacterial Blight caused by *Xanthomonas oryzae* pv. *oryzae*

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Keywords

Xanthomonas oryzae pv. oryzae, bacterial blight, resistance genes, disease scoring, rice

Article Info

Accepted:
24 August 2019
Available Online:
10 September 2019

A B S T R A C T

India is among the topmost rice producers and consumers in the world. Rice crop is susceptible to various bacterial diseases and one such commonly known disease is Bacterial Blight (BB) caused by the pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and is known to severally impact rice crop yield. Rice variety HKR-47 is widely popular amongst rice farmers and consumers in Haryana because of its high yield, medium slender grains, and excellent cooking and eating qualities, however, HKR-47 exhibits less endurance to BB. The aim of the study conducted at CCS Haryana Agricultural University was to investigate the genetic potential of BC_3F_3 pyramided rice genotypes (cross HKR-47 x IRBB-60) having resistance genes (*Xa21*, *xa13* and *xa5*). These genotypes were tested for virulence against BB under artificial conditions using Clip method of artificial inoculation. On average, five leaves per plant were inoculated and visual scoring was done after 14 days. Rating of disease reaction was based on a 0-9 scale of the standard evaluation system (SES) for rice. Rice genotypes with all three genes exhibited relatively low mean lesion length compared to single or double combinations thus establishing higher resistance of three-gene genotypes to BB. The lines obtained in our study can be used as genetic resources for BB resistance in breeding programs that will be paving the way for an environmentally-friendly means to achieve a better disease management.

Introduction

Bacterial Blight caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the oldest known bacterial disease of rice (*Oryza sativa* L.) in Asia. It is a major pathogen that adversely impacts rice production, especially in irrigated and rainfed lowland agricultural production systems (Mew *et al*., 1992). BB causes yield losses ranging from 74% to 81% (Srinivasan and Gnanamanickam, 2005) in severe conditions, depending on the stage of the crop, cultivar susceptibility and the environmental conditions (Noh *et al*., 2007). Bacterial Blight

can cause damage at vegetative and reproductive stages of rice plants. *Xoo* invades the plant through wounds or water pores. Lesions with wavy margins start from the tip of the leaf as the water pores are located at the margins of upper parts of the leaf. These water-soaked lesions enlarge in size, turn yellow and ultimately lead to the death of plant (Nino-Liu *et al*., 2006).

Systemic nature of the disease, lack of effective chemical control measures (Devadath, 1989) and the concern over health hazards of pesticides have limited the utilization of chemical control agents (Guillebeau, 1998). Resistance from the host plant is known to offer the most effective, economical and environmentally safe option for management of BB pathogen in rice (Khush *et al*., 1989). Long-term cultivation of rice varieties carrying a single resistance gene has resulted in a significant shift in pathogen-race frequency and consequent breakdown of resistance (Mew *et al*., 1992). Pyramiding of multiple resistance genes in the background of modern high yielding varieties is a tangible solution to resistance breakdown.

Gene pyramiding aims to assemble desirable genes from multiple parents into a single genotype. It provides a broad-spectrum resistance which is an economical and effective method for BB management (Babujee and Gnanamanickam, 2000). Major resistance genes, such as *Xa4*, *xa5*, *Xa7*, *xa13* and *Xa21* have been incorporated into rice cultivars, in order to develop new resistant varieties (Perumalsamy *et al*., 2010). Most of these genes follow the classic gene-for-gene concept for the race-specific interaction between rice and *Xoo* (Flor, 1971). Some resistance genes are effective only in adult plants, while others are effective at all stages of growth. *Xa21* mediated resistance gene expressed resistance at the seedling stage whereas *xa5* and *Xa4* gene could confer resistance at all growth stages (Adhikari *et al*., 1995; Garris *et al*., 2003; Arif *et al*., 2008). Some genes confer resistance to a broad spectrum of *Xoo* races, whereas others do so against only one or a few races. e.g. *xa5* and *Xa4* gene could confer broad spectrum of resistance to *Xoo* isolates whereas *xa13* gene shows broad resistance only in adult plants (Sidhu *et al*., 1978). The probability of simultaneous pathogen mutations for virulence to defeat two or more effective genes is much lower than with a single gene (Mundt, 1990) and thus this study aims to establish the effectiveness of multiple resistance genes against BB.

Materials and Methods

The study material consisted of BB resistance genes pyramided BC_3F_3 genotypes (selected on the basis of molecular marker analysis) derived from the cross between BB susceptible HKR-47 (recurrent parent) and BB resistant IRBB-60 (donor parent).

Collection, isolation and maintenance of *Xoo* isolate

Infected rice leaves showing bacterial blight symptoms were collected from the BB infected leaves from the fields of RRS, Kaul (Figure 1 (a)). These leaves were surface-sterilized with 2% sodium hypochlorite for 1 minute 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 then were streaked for single-colony isolation on PSA plates (Figure 1 (b)). *Xanthomonas oryzae* pv. *oryzae* was circular, smooth, convex, opaque and whitish yellow at first and turned straw yellow later as identified on PSA plates. Well separated colonies of the isolate were picked up and streaked on PSA media in laminar flow (Table 1). The *Xoo* isolate was multiplied and

maintained on Peptone Sucrose Agar (PSA) plates kept in the growth room at 28°C for 72 hours. The culture so obtained was stored in the refrigerator at 4ºC. For inoculation, the inoculum was prepared by suspending the bacteria in sterile distilled water prior to the inoculation period. The absorbance value (590 mm) was adjusted to 1 to give a bacterial suspension with a concentration of approximately 10^9 cfu/ml (in log phase).

The genotypes, along with the control (un-inoculated seedlings), were inoculated with the *Xoo* isolate. The plants were clip inoculated at the maximum tillering stage. The leaf blades were inoculated by clipping with *Xoo* suspension infected scissors at 3 cm below the leaf tips (Kauffman *et al*., 1973). On an average, five leaves per plant were inoculated and were regularly observed for the symptoms appearance. The disease severity was measured 14 days after inoculation (Figure 2) and rating the disease reaction was done on a 0-9 scale (Table 2) of the SES for rice (Anonymous, 1996).

Disease Measurement

Percent disease incidence (%DI) was calculated according to (Gnanamanickam *et al*., 1999) formula as follows:

$$\% \text{ Disease incidence} = \frac{\text{Total lesion length}}{\text{Total leaf length}} \times 100$$

Disease Scoring

On the basis of mean lesion length, the genotypes were grouped into different categories of resistance and susceptibility using standard evaluation system (SES) developed at International Rice Research Institute (IRRI), Philippines.

Results and Discussion

The positive BC_3F_3 lines were evaluated for their resistance to bacterial blight in the field and under net house conditions using the *Xanthomonas oryzae* strain isolated from the BB infected fields of RRS, Kaul. One hundred twenty BC_3F_3 genotypes (Tables 3 and 4) with single or multiple type BB resistance genes (*Xa21*, *xa13* and *xa5*) along with the parents were evaluated for their resistance to bacterial blight in the field as well as in net house using the *Xanthomonas oryzae* strain. The pyramided lines along with the control were inoculated using a bacterial suspension of 10^9cells/ml. The ten three-gene positive BC_3F_3 plants (lesion length range 0.50-0.90 cm) derived in the study from the cross, were found to be almost as effective against the virulent *Xoo* strain as the donor parent IRBB-60 (mean lesion length of 0.50 cm). These ten three-gene positives (*Xa21*, *xa13* and *xa5*) BC_3F_3 plants showed a mean lesion length of 0.54 cm. On screening for BB resistance, the mean lesion length among positive lines varied from 0.50 cm to 10.30 cm. Fifty lines having *Xa21*/*xa13* genes (mean lesion length of 4.46 cm), eight lines having *Xa21*/*xa5* (mean lesion length of 4.60 cm) and four lines having *xa13*/*xa5* (mean lesion length of 5.1 cm) were found to be resistant or moderately resistant to the BB disease. However, the lines having *Xa21* gene alone (mean lesion length of 5.30 cm) were found to be more resistant than the lines with *xa5* gene alone (mean lesion length of 7.25 cm) or *xa13* gene alone (mean lesion length of 10.30 cm) (Figure 3). The lines with two-gene combination had a higher level and broader spectrum of resistance than parental lines or lines with a single gene (Tables 4 and 5). The results indicated that the genes in combinations were more effective and durable against the pathogen than a single gene and that there is some kind of quantitative complementation with the presence of multiple resistance genes

which have an additive effect on the overall level of resistance.

Through gene interaction and complementation, lines with pyramided genes were found to increase resistance quantitatively and provide a broader spectrum of resistance over those conferred by single genes (Yoshimura *et al*., 1995; Singh *et al*., 2001).

Furthermore, the lines having *Xa21* resistant gene alone were found to be more resistant to BB disease than the lines having *xa13* or *xa5* alone. *Xa21* was the most effective, followed by *xa*5. Resistance gene *xa13* was the least effective against *Xoo*. The study conducted by Nikita *et al*. (2016) showed that individually, *xa5* and *Xa21* were more effective resistance genes than *xa13*. This is in agreement with those reported in our study. The locus, *Xa21*, was found to confer resistance to all known *Xanthomonas oryzae* pv. *oryzae* races in India and Philipines (Khush *et al*., 1990 and Ikeda *et al*., 1990). The locus may encode a single gene product that specifies *Xa21* resistance to multiple pathogen isolates, or the locus may be composed of a cluster of tightly linked genes, each of which recognizes a unique isolate-specific determinant.

The higher lesion lengths observed in some combinations could be the result of recombination between marker locus and the target gene. This is more likely for *xa13* since the linked marker RG136 is 3.8 cM away from the resistant gene as compared to pTA248 and RG556, the gene sequence based markers for *Xa21* and *xa5*, respectively.

With the availability of a gene based marker for *xa13* (cited in Singh *et al*., 2011), the transfer can be done with higher precision.

Rajpurohit *et al.* (2010) also presented the similar results by recording disease reaction in forty BC_2F_3 progenies of Type 3 basmati containing individual *xa13* and *Xa21* genes or combination of both under artificial inoculation conditions using mixture of seven *Xoo* isolates. Their results showed that the progenies having both the resistance genes *Xa21* and *xa13* were highly resistant to BB disease than the progenies having individual resistance genes. However, progenies having *xa13* gene alone were found to be more effective than the progenies having only *Xa21* gene. But in the present study, the BC_3F_3 plants having *xa13* gene alone were less effective than the plants having *Xa21* gene.

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

Sucrose	**5.0 g**
Sodium glutamate	1.0 g
Ferrous sulphate	0.25 g
Yeast extract	2.5 g
Peptone	10.0 g
Agar	15.0 g
pH	6.0

Table.2 Disease rating using 0-9 scale

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)

Table.3 Number of BC_3F_3 plants with single or multiple resistance gene(s)

S. No.	Gene combinations	No. of BC_3F_3 plants	BC_3F_3 plants (Number Lines)
1	***Xa21/Xa21xa13/xa13xa5/xa5***	10	G1-8,12,13, 15; G2-3, 6; G3-11, 13;G5-13, 17
2	***Xa21/Xa21xa13/xa13Xa5/Xa5***	50	G1-1, 6, 7, 9, 16, 19,20; G2-2, 4, 5, 8, 9, 12, 14, 20; G3- 1, 3, 5, 6, 7, 9, 14, 15, 16, 17, 19, 20; G4-1, 2, 3, 4, 5, 9 ,10, 11; G5- 2, 6, 7, 10, 11, 12, 16, 18,19; G6-2, 4, 5, 12, 18, 20
3	***Xa21/Xa21Xa13/Xa13xa5/xa5***	08	G1-2; G2-11,16; G4-6, 8; G5-9, 14; G6-14
4	***xa21/xa21xa13/xa13xa5/xa5***	04	G4-18; G5-15; G6-17, 19
5	***Xa21/Xa21Xa13/Xa13Xa5/Xa5***	27	G1-3, 4, 5, 10, 14, 18 ; G2-1, 7, 13, 15, 17, 18; G3- 18; G4- 7, 14, 16, 17; G5-3; G6- 1, 3, 6, 8, 9, 10, 11, 13, 15
6	***xa21/xa21xa13/xa13Xa5/Xa5***	17	G1-11, 17; G2-10; G3-2, 4, 8, 12, ; G4- 12, 13, 15, 19, 20; G5- 1, 4, 5, 8, 20
7	***xa21/xa21Xa13/Xa13xa5/xa5***	04	G2-19, G3-10, G6-7, 16

Table.4 Disease reaction of BC_3F_3 rice genotypes (containing one, two or three BB resistance genes) to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) (Nine point rating scale for scoring of bacterial blight disease)

S.No.	Parents and BC_3F_3 genotypes	*Xa21*	*xa13*	*xa5*	No. of R genes	Disease incidence (%)	Disease rating	Reaction category
1	IRBB-60	+	+	+	3	0.5	0	HR
2	HKR-47	-	-	-	0	76.6	9	HS
3	G1-1	+	+	-	2	11.0	3	MR
4	G1-2	+	-	+	2	15.0	3	MR
5	G1-3	+	-	-	1	12.5	3	MR
6	G1-4	+	-	-	1	10.5	3	MR

7	G1-5	+	-	-	1	13.7	3	MR
8	G1-6	+	+	-	2	8.7	1	R
9	G1-7	+	+	-	2	17.0	3	MR
10	G1-8*	+	+	+	3	0.9	0	HR
11	G1-9	+	+	-	2	7.9	1	R
12	G1-10	+	-	-	1	11.5	3	MR
13	G1-11	-	+	-	1	14.4	3	MR
14	G1-12*	+	+	+	3	0.8	0	HR
15	G1-13*	+	+	+	3	0.7	0	HR
16	G1-14	+	-	-	1	37.0	5	MS
17	G1-15*	+	+	+	3	68.0	0	HR
18	G1-16	+	+	-	2	13.3	3	MR
19	G1-17	-	+	-	1	32.0	5	MS
20	G1-18	+	-	-	1	30.4	5	MS
21	G1-19	+	+	-	2	18.9	3	MR
22	G1-20	+	+	-	2	12.6	3	MR
23	G2-1	+	-	-	1	11.7	3	MR
24	G2-2	+	+	-	2	7.0	1	R
25	G2-3*	+	+	+	3	0.8	0	HR
26	G2-4	+	+	-	2	13.6	3	MR
27	G2-5	+	+	-	2	16.6	3	MR
28	G2-6*	+	+	+	3	0.8	0	HR
29	G2-7	+	-	-	1	51.3	7	S
30	G2-8	+	+	-	2	22.0	3	MR
31	G2-9	+	+	-	2	27.0	3	MR
32	G2-10	-	+	-	1	32.5	5	MS
33	G2-11	+	-	+	2	52.3	7	S
34	G2-12	+	+	-	2	41.4	5	MS
35	G2-13	+	-	-	1	11.5	3	MR
36	G2-14	+	+	-	2	12.6	3	MR
37	G2-15	+	-	-	1	9.8	1	R
38	G2-16	+	-	+	2	14.2	3	MR
39	G2-17	+	-	-	1	8.6	1	R
40	G2-18	+	-	-	1	17.2	3	MR
41	G2-19	-	-	+	1	33.5	5	MS
42	G2-20	+	+	-	2	10.9	3	MR
43	G3-1	+	+	-	2	12.6	3	MR
44	G3-2	-	+	-	1	31.7	5	MS
45	G3-3	+	+	-	2	12.9	3	MR
46	G3-4	-	+	-	1	13.6	3	MR
47	G3-5	+	+	-	2	13.9	3	MR
48	G3-6	+	+	-	2	17.2	3	MR
49	G3-7	+	+	-	2	8.3	1	R
50	G3-8	-	+	-	1	14.4	3	MR
51	G3-9	+	+	-	2	14.9	1	MR
52	G3-10	-	-	+	1	15.9	3	MR
53	G3-11*	+	+	+	3	0.7	0	HR

54	G3-12	-	+	-	1	14.9	3	MR
55	G3-13*	+	+	+	3	0.8	0	HR
56	G3-14	+	+	-	2	9.2	1	R
57	G3-15	+	+	-	2	13.6	3	MR
58	G3-16	+	+	-	2	6.9	1	R
59	G3-17	+	+	-	2	8.3	1	R
60	G3-18	+	-	-	1	12.9	3	MR
61	G3-19	+	+	-	2	7.9	1	R
62	G3-20	+	+	-	2	8.6	1	R
63	G4-1	+	+	-	2	13.3	3	MR
64	G4-2	+	+	-	2	9.1	1	R
65	G4-3	+	+	-	2	13.7	3	MR
66	G4-4	+	+	-	2	6.3	1	R
67	G4-5	+	+	-	2	12.7	3	MR
68	G4-6	+	-	+	2	12.2	3	MR
69	G4-7	+	-	-	1	7.9	1	R
70	G4-8	+	-	+	2	13.4	3	MR
71	G4-9	+	+	-	2	13.6	3	MR
72	G4-10	+	+	-	2	17.9	3	MR
73	G4-11	+	+	-	2	16.6	3	MR
74	G4-12	-	+	-	1	33.9	5	MS
75	G4-13	-	+	-	1	34.5	5	MS
76	G4-14	+	-	-	1	8.2	1	R
77	G4-15	-	+	-	1	31.9	5	MS
78	G4-16	+	-	-	1	8.4	1	R
79	G4-17	+	-	-	1	5.3	1	R
80	G4-18	-	+	+	2	12.3	3	MR
81	G4-19	-	+	-	1	36.2	5	MS
82	G4-20	-	+	-	1	35.9	5	MS
83	G5-1	-	+	-	1	30.2	5	MS
84	G5-2	+	+	-	2	11.9	3	MR
85	G5-3	+	-	-	1	3.9	1	R
86	G5-4	-	+	-	1	13.6	3	MR
87	G5-5	-	+	-	1	19.9	3	MR
88	G5-6	+	+	-	2	18.6	3	MR
89	G5-7	+	+	-	2	11.4	1	MR
90	G5-8	-	+	-	1	35.3	5	MS
91	G5-9	+	-	+	2	18.5	3	MR
92	G5-10	+	+	-	2	11.3	1	MR
93	G5-11	+	+	-	2	13.8	1	MR
94	G5-12	+	+	-	2	12.5	1	MR
95	G5-13*	+	+	+	3	0.6	0	HR
96	G5-14	+	-	+	2	11.9	3	MR
97	G5-15	-	+	+	2	17.2	3	MR
98	G5-16	+	+	-	2	14.5	3	MR

99	G5-17*	+	+	+	3	5.5	0	HR
100	G5-18	+	+	-	2	13.5	3	MR
101	G5-19	+	+	-	2	7.8	1	R
102	G5-20	-	+	-	1	8.4	1	R
103	G6-1	+	-	-	1	9.4	1	R
104	G6-2	+	+	-	2	11.2	3	MR
105	G6-3	+	-	-	1	10.9	3	MR
106	G6-4	+	+	-	2	11.9	3	MR
107	G6-5	+	+	-	2	13.4	3	MR
108	G6-6	+	-	-	1	15.6	3	MR
109	G6-7	-	-	+	1	32.8	5	MS
110	G6-8	+	-	-	1	12.5	3	MR
111	G6-9	+	-	-	1	13.6	3	MR
112	G6-10	+	-	-	1	14.4	3	MR
113	G6-11	+	-	-	1	15.9	3	MR
114	G6-12	+	+	-	2	16.2	3	MR
115	G6-13	+	-	-	1	12.6	3	MR
116	G6-14	+	-	+	2	12.6	3	MR
117	G6-15	+	-	-	1	12.9	3	MR
118	G6-16	-	-	+	1	30.5	5	MS
119	G6-17	-	+	+	2	14.0	3	MR
120	G6-18	+	+	-	2	15.9	3	MR
121	G6-19	-	+	+	2	16.7	3	MR
122	G6-20	+	+	-	2	14.5	3	MR

* indicates three-gene positive genotypes

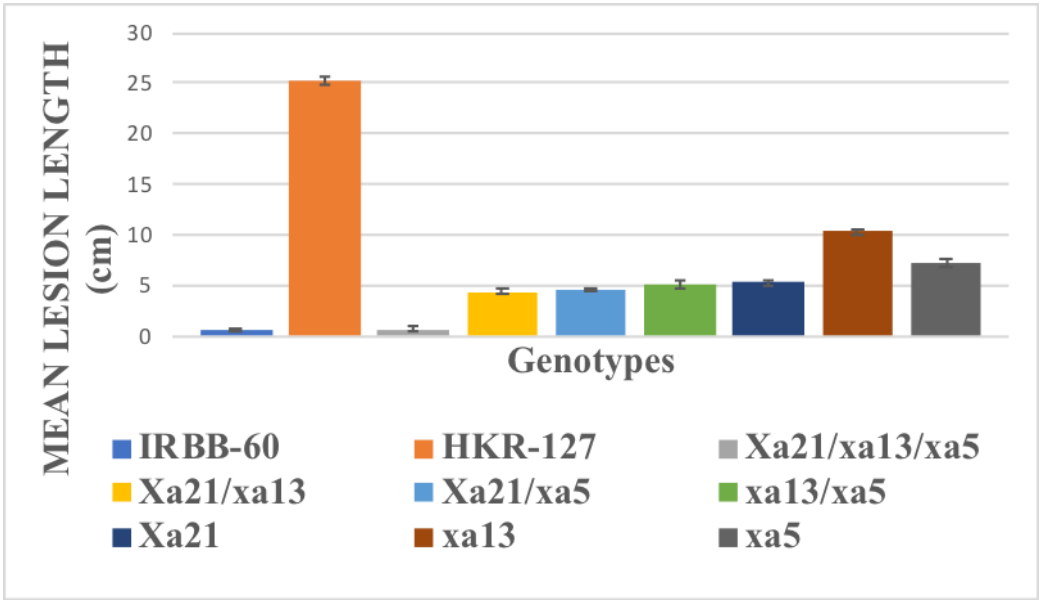
Table.5 Categorizing the number of BC_3F_3 genotypes to BB disease response using 0-9 scale of disease rating

Infection (%)	Score	Host response	Range of % leaf area infected	Number of plants
0	0	Highly resistant (HR)	0.6-0.9	11
>1 -10	1	Resistant (R)	3.9-9.8	21
>10 -30	3	Moderately resistant (MR)	10.5-27	67
>30 -50	5	Moderately susceptible (MS)	30.4-41.4	20
>50 -75	7	Susceptible (S)	51.3-52.3	2
>75 -100	9	Highly susceptible (HS)	76.6	1

Fig.1 (a) Bacterial Blight infected leaves; (b) Purified culture of *Xanthomonas oryzae* pv. *oryzae* on PSA medium

Fig.2 Disease scoring after 14 days of inoculation (a) Highly Susceptible genotype (b) Highly Resistant genotype

Fig.3 Disease reaction of the donor parent, susceptible parent and pyramid lines (*Xa21*, *xa13* and *xa5*)



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How to cite this article:

Kirti Mehta, Nikita Baliyan, Rahul Kumar Meena and Shikha Yashveer 2019. Evaluation of Resistance of Rice Genotypes (Derived from the Cross between HKR-47 and IRBB-60) against Bacterial Blight caused by *Xanthomonas oryzae* pv. *oryzae*. *Int.J.Curr.Microbiol.App.Sci*. 8(09): 2755-2765. doi: https://doi.org/10.20546/ijcmas.2019.809.317