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Assessment of virulence of bacterial blight (*Xanthomonas oryzae* pv *oryzae* (Ishiyama) Swings *et al.*) of rice (*Oryza sativa* L.) and identification of genotypes with high resistance

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Abstract

Bacterial blight (BB) of rice is the second most devastating disease that causes substantial yield loss. Study of pathogenic variability and identification of resistance genes are key factors in breeding against this disease. Five isolates collected from different parts of northwestern India were screened against eight aromatic genotypes to identify variability in virulence. Most virulent isolate was used to screen forty eight rice genotypes collected from different regions of Bihar, Jharkhand and UP to identify resistant sources. All the aromatic genotypes were found to be either moderately or highly susceptible against all the isolates with significant differences in disease progress. Isolate 5 was found to be most virulent against the aromatic lines while isolate 4 was least virulent. Isolate 5 was used to screen forty-eight genotypes. Thirteen lines showed high resistance against the highly virulent isolate. Inheritance of resistance in these rice lines should, therefore, be investigated further.

Key words: Basmati rice, bacterial blight, virulence, landrace

Introduction

Rice is undoubtedly the most important staple food grain in the world. Demand for rice in India is projected at 128 million tonnes by the year 2012, which requires increase in productivity to at least 3 tonnes/ha from the present 2 tonnes/ha [1]. Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swings *et al.* (*Xoo*) is the second most important disease of rice after blast and most important bacterial disease in terms of economic loss. It causes an annual loss of 20-30% in Japan and 6-60% in India. It caused major epidemic in Punjab, Haryana and Western Uttar Pradesh in 1979 and 1980 and caused complete destruction of crop [2]. Recently, the disease has caused a severe epidemic in the Palghat district of Kerala [3]. As chemical methods of control are costly and hazardous to environment, resistance breeding is the wisest option to tackle the disease.

Study on pathogenic variability as well as identification of resistance genes are two key objectives in resistance breeding. A common approach in differentiating pathogenicity of identified *Xoo* isolates is to employ differential lines or near isogenic lines carrying different resistant genes. Five *Xoo* isolates, collected from Punjab and Haryana were characterized by employing such methods, which identified isolate 5 as most virulent [4]. Although near isogenic lines are quite effective in differentiating virulence pattern, they can not be employed to measure relative rate of disease development, which is an important parameter in differentiating *Xoo* isolates. This would also help in pointing out more aggressive *Xoo* isolates that have the potential to cause greater damage. For this purpose, a set of susceptible genotypes that would not restrict disease development is required. The zone is famous for Basmati rice, which is highly susceptible to bacterial blight. An experiment was, therefore, designed to identify aggressive strains among five *Xoo* isolates using eight Basmati genotypes.

The pathogenic variability accounts for susceptibility of resistant lines identified in one location in other rice growing areas. Such genes for resistance to specific races may not be effective in all the regions. It has been observed that none of the designated resistance genes produce resistance to most isolates found in Punjab and Haryana; therefore these genes are of little use in practical resistance breeding [5]. The probability of identifying resistant sources is more in traditional rice cultivars and land races than in the modern high yielding varieties with widespread cultivation, as the modern advanced genotypes have narrow gene pool while the land races are rich in genetic diversity. Indian traditional rice germplasms may contain resistance genes that may be more suitable to counteract virulent Indian pathotypes more effectively

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than the resistance genes identified abroad. Considering this, forty eight genotypes were tested against the most virulent and aggressive *Xoo* isolate for identifying new resistance sources.

Materials and methods

Establishment and maintenance of pure culture: Five isolates were collected from Basmati growing regions of Punjab and Haryana. Live disease samples collected in the form of infected green leaves were surface sterilized and thin sections were taken after dipping in sterile distilled water. A bacterial solution was then prepared by crushing the infected leaf sections in drops of sterile water on sterile slides for each isolate separately. For inoculation subculturing was done in test tubes with slanted medium and bacterial cultures were incubated at 28-30°C. Inoculum was prepared by suspending each pure culture in sterile distilled water. The concentration of bacterial suspension was adjusted to 10^9 cfu/ml during inoculation.

Field screening: Three most popular basmati cultivars, *viz.*, Basmati 370, Taraori Basmati and Pusa Basmati 1 and five enhanced Basmati germplasm, *viz.* Pusa 1301, Pusa 1302, Pusa 1302-3-3-2-4-1-1, Pusa 1302-4-1-1-10-1 and Pusa 1121 were grown in the *kharif* season of 2002 in a randomized block design with four replications with a plot size of 5 m × 3 m. Row to row spacing of 25 cm and plant to plant distance of 20 cm was given during transplanting. Standard agronomic management practices were followed to raise a healthy crop. The plants were inoculated with the 5 different isolates of *Xoo* at maximum tillering stage following the leaf inoculation method [6]. Each isolate was used on 5 random plants and differentially coloured tags were used to differentiate between inoculated plants of each isolate. The disease score was taken 14 days after inoculation. Plants were classified as resistant and susceptible following Ogawa [7].

For identification of resistance lines a set of 48 rice land races collected from different regions of the states of Bihar, Jharkhand and U. P. were screened with the most virulent isolate identified from the previous study. A layout of randomized complete block design with four replications was followed to grow these genotypes in the field with a plot size of 2 m × 1 m. For identification of resistance, a line having lesion length less than 4 cm was considered highly resistant.

Results and discussion

The reactions of five isolates of *Xoo* on eight aromatic rice cultivars are presented in Table 1. Although all the genotypes were found to be susceptible, the degree of susceptibility, as measured by lesion length progress varied significantly from each other. Among the lines tested, the released Basmati cultivars showed more susceptibility to bacterial blight than the advanced breeding lines. Two released Basmati varieties, Taraori Basmati and Pusa Basmati 1 were found to show very high degree of susceptibility against all the isolates. Significance of mean square due to *Xoo* isolates proves that the aggressive natures of the selected *Xoo* isolates are different (Table 2, 3). It clearly indicates that considerable variability is present among the isolates regarding their infectivity and disease progress on the Basmati genotypes. Lowest mean lesion length was expressed by Pusa 1302-3-3-2-4-1-1 with a value of 12.13 cm. Five out of the eight cultivars tested showed lower lesion length than the mean.

Table 1. Reactions of five bacterial isolates on eight Basmati quality lines

Genotype	Mean lesion length (cm)				
	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5
Basmati 370	13.90	15.53	11.50	11.03	15.83
Pusa Basmati 1	21.30	23.40	18.40	17.17	25.60
Pusa 1301	12.97	14.83	13.53	14.57	15.50
Pusa 1302-3-3-2-4-1-1	10.90	12.93	12.50	11.13	13.17
Pusa 1302-4-1-1-10-1	17.30	17.40	16.67	14.77	17.43
Pusa 1302	12.80	14.40	15.57	13.20	14.63
Taraori Basmati	23.70	23.23	18.33	20.13	25.60
Pusa 1121	13.47	15.73	14.33	11.73	14.27

Basmati 370 was less susceptible to the isolate 3 and isolate 4. The higher susceptibility of the released Basmati cultivars may be attributed to their long cultivation period in the farmers' field, during which they have got more exposure to pathogen attack and more virulent strains have evolved against them, while the advanced breeding lines have been developed in Delhi region, which does not provide conducive environment for the disease. In the present experiment Basmati 370 was found to be moderately susceptible

Table 2. ANOVA of lesion length produced by *Xoo* isolates on eight Basmati quality genotypes

Isolates	df	MS	Pr > F	Mean	LSD
1	7	62.004	< 0.001	15.79	1.89
2	7	47.76	< 0.001	17.18	2.34
3	7	20.05	< 0.001	15.10	1.76
4	7	30.42	< 0.001	14.22	1.37
5	7	74.97	< 0.001	17.75	2.34

Table 3. ANOVA for lesion length produced by five *Xoo* isolates on eight Basmati quality genotypes

Source	df	MS	Pr > F	LSD
Isolate	4	51.01	< 0.001	0.63
Replication	2	8.45	0.036	-
Genotype	7	1462.36	< 0.001	0.80
Genotype × Isolate	28	184.10	< 0.001	-

Table 4. Disease reactions of *Xoo* isolate 5 on forty eight landraces of rice

Sl. No.	Germplasm	Source	Mean lesion length (cm)	Reaction
1	Asimpa	Palamu, Jharkhand	13.25	MS
2	Baba	Tilhar, UP	3.75	R
3	Badshabhog	Palamu, Jharkhand	15.25	S
4	Baghpanjar-1	Sitamarhi, Bihar	9.75	MR
5	Baghpanjar-2	Sitamarhi, Bihar	1.25	R
6	Basmati	Tilhar, UP	13.25	MS
7	Barhasal-1	Palamu, Jharkhand	2.12	R
8	Barhasal-2	Palamu, Jharkhand	14.50	MS
9	Bachakalamdani-1	Sarguja, MP	6.30	MR
10	Bachakalamdani-2	Sarguja, MP	20.50	S
11	Bejhari	Sitamarhi, Bihar	16.00	S
12	Bhoosi	Palamu, Jharkhand	13.50	MS
13	Bhuthi	Sitamarhi, Bihar	9.35	MR
14	Bhasar	Sitamarhi, Bihar	4.75	R
15	Bhama	Sarguja, MP	16.25	S
16	Bhantaphool-1	Palamu, Jharkhand	26.25	S
17	Bhantaphool-2	Palamu, Jharkhand	15.75	S
18	Dahiya	Ranchi, Jharkhand	2.00	R
19	Danigoda-1	Palamu, Jharkhand	29.50	S
20	Dhoki	Palamu, Jharkhand	3.75	R
21	Dubraj-1	Sarguja, MP	18.00	S
22	Dubraj-2	Sarguja, MP	14.25	MS
23	Hindmauri	Palamu, Jharkhand	25.00	S
24	Jhilli-1	Sarguja, MP	8.75	MR
25	Jhilli-2	Sarguja, MP	5.38	R
26	Kalajeera-A	Palamu, Jharkhand	5.00	R
27	Kalajeera-B	Palamu, Jharkhand	3.75	R
28	Kalamdani	Sarguja, MP	3.88	R
29	Karma-1	Sitamarhi, Bihar	2.13	R
30	Karma-2	Sitamarhi, Bihar	6.00	MR
31	Kherka Kuchi	Sarguja, MP	22.00	S
32	Mahacheenavar	Sarguja, MP	9.75	MR
33	Malkhuji-3	Palamu, Jharkhand	18.75	S
34	Pharka Pakh	Sarguja, MP	12.75	MS
35	Rani Kajar-1	Palamu, Jharkhand	21.25	S
36	Rani Kajar-2	Palamu, Jharkhand	18.50	S
37	Ramdi	Palamu, Jharkhand	13.00	MS
38	Ramdi-2	Palamu, Jharkhand	24.00	S
39	Ruchi-1	Sarguja, MP	19.00	S
40	Ruchi-2	Sarguja, MP	21.25	S
41	Safri-1	Palamu, Jharkhand	20.00	S
42	Sathi-2	Tilhar, UP	11.13	MS
43	Sheetalbhog	Sarguja, MP	8.25	MR
44	Sheetalbhog-1	Sarguja, MP	3.13	R
45	Sheetalbhog-2	Sarguja, MP	6.50	MR
46	Sikki	Sarguja, MP	8.25	MR
47	Silang	Palamu, Jharkhand	5.63	R
48	Vuripa	Sarguja, MP	16.75	S

to isolate 1, 3, and 4 and showed higher susceptibility to isolate 2 and 5, while Taraori Basmati was highly susceptible to all the isolates. The difference between lesion length of Taraori Basmati and Pusa Basmati 1 was found to be non significant for isolate 2 and isolate 5, while for other *Xoo* isolates they differed significantly. Taraori Basmati exhibited higher susceptibility to *Xoo* attack.

Highest mean lesion length (17.75 cm) was produced by isolate 5, although difference between mean lesion length produced by isolate 5 and isolate 2 was found to be non-significant. These two isolates, therefore, may be considered more aggressive than others. Lowest mean lesion length of 14.22 cm was observed when the host genotypes were screened with isolate 4. This isolate, therefore, is less aggressive and may not be able to cause large-scale damage of crop.

The isolates were grouped by a clustering technique according to their aggressiveness on the aromatic Basmati quality rice genotypes. Although the clustering is based on lesion length alone, it gives a preliminary idea among the relationship of different isolates of *Xoo*. Another advantage is that unlike t-test, which considers mean lesion length only, the clustering technique involves observations on all the genotypes and therefore provides a better understanding of relationship between different isolates. In the present case, the dendogram output did not deviate from the results oft-test. Isolate 2 and 5 were found to be more related to each other in terms of virulence than the other isolates. Isolate 4, which showed least virulence was situated farthest from isolate 2 and isolate 5 in the cluster. Considering the high virulence and aggressiveness of *Xoo* isolate 5, the landraces were screened for resistance against this isolate [4].

A grand mean lesion length of 12.27 cm was observed after screening the germplasms with isolate five. Genotypic differences for disease progress were found to be highly significant (Table 5). Danigoda-1 recorded highest susceptibility with a mean lesion length of 29.5 cm, while Baghpanjar-2 exhibited lowest disease development with a mean lesion length of 1.25 cm. While Danigoda-1 was found to be significantly different from all other genotypes differences between the genotypes with low lesion length viz. Baghpanjar-2, Dahiya, Barhasal-1, Karma-1, Sheetabhog-1, Dhoki, Baba and Kalajeera-B were found to be non-significant. Out of the forty eight lines tested thirteen were found to have lesion length less than 6 cm and were classified

Table 5. ANOVA for disease reaction of rice germplasms screened with isolate 5

Source	df	Mean square	Pr > F	LSD
Replication	3	1.61	0.69	
Germplasm	47	10397.85	< 0.001	2.525

as highly resistant (Table 4). Eighteen lines showed high susceptibility to the disease. Among the rest eight were moderately susceptible while another nine were found to be moderately resistant. Among the thirteen resistant lines, nine had a lesion length less than 4 cm that indicates nearly immune reaction. We, therefore report presence of new sources of highly effective resistance genes against bacteria blight of rice. Mode of inheritance of these lines should be studied further for enriching the breeding efforts for resistance against *Xoo*. Genes having simple inheritance pattern would be more useful than with complex or epistatic inheritance. As all the Basmati lines tested in the present experiment were susceptible against bacterial blight, effective resistance genes therefore should be transferred to the Basmati background from resistant donor lines.

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