Pak. J. Bot., 43(6): 3021-3023, 2011.

EVALUATION OF RICE GERMPLASM AGAINST *XANTHOMONAS ORYZAE* CAUSING BACTERIAL LEAF BLIGHT

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Abstract

Bacterial blight (BB) caused by *Xanthomonas oryzae* pv *oryzae* is the most destructive disease of rice in Pakistan due to its high epidemic potential. Forty seven rice accessions comprising 6 crosses, 8 wild rice, 2 commercial varieties, 23 isogenic lines, 7 new plant type and 1 mutant, were evaluated against 25 isolates (X01, X02, X03, X04, X05, X06, X07, X08, X09, X010, , X011, X012, X013, X014, X015, X016, X017, X018, X019, , X020, X0139, X0140, X0143, X0146, X067) in bulk form under heavy inoculum load in glass house. Data were recorded at 15 days after inoculation on 0-4 rating scale. Interspecific crosses showed a range from 1.0 to 13 cm, wild rice alone showed range from 1.0 to 12cm, isogenic lines of rice showed range from 1.2 to 29.8 cm, while new plant type, mutant and commercial varieties varied within the range 1.0 to 12.0cm, 6.6, and 16.8 to 18.4 cm, respectively. Resistance against bacterial leaf blight isolates was observed in IR6 x *Oryza nivara*, *O. longistminata*, *O. punctata*, *O. nivara* and IRBB24 (isogenic line). Bas.385 and KS282, the cultivated rice were susceptible to all isolates. Use of resistant wild species viz., *O. nivara*, *O. longistminata* and *O. punctata* are therefore recommended for transgressing bacterial blight resistance to the cultivated rice.

Introduction

Bacterial blight of rice caused by *Xanthomonas oryzae* pv *oryzae (Xoo)* is the most destructive disease of rice in Pakistan and can reduce the yield up to 20 to 30% (Ou, 1985). The disease reduces grain yield to varying levels depending on the stage of the crop, degree of cultivar susceptibility and a great extent to the conduciveness of the environment in which it occurs. Bacterial blight appears on leaves of young plants, as pale-green to grey-green water-soak streaks near the leaf tip and margins. These lesions coalesce and become yellowish-white with wavy edges. Eventually, the whole leaf may become whitish or grayish and then dies. Leaf sheaths and culms of highly susceptible cultivars may also be attacked. Systemic infection, known as kresek (Reddy, 1984), results in desiccation of leaves and death, particularly of young transplanted plants. In older plants, the leaves become yellow and then die. The objective of this study was to identify resistance gene against bacterial blight which may be used for developing new varieties with genes conferring resistance to bacterial blight.

Materials and Method

Forty seven rice (*Oryza* L.) accessions comprising 6 interspecific crosses, 8 wild collections, 2 commercial varieties, 23 isogenic lines, 7 new plant type and 1 mutant, collected from different sources were tested for resistance against *Xoo*, which cause bacterial blight in rice. Nursery of the germplasm was prepared in field beds and later on transplanted in glass house. Each line was transplanted in individual pot and all the cultural practices were applied to maintain proper growth condition. *Xanthomonas oryzae* pv *oryzae* isolates were used to test the rice germplasm resistance.

Isolation and multiplication of *Xoo*: Sixty diseased samples of rice leaves were collected from different areas of the Punjab and used for the isolation of *Xoo* (Akhtar & Rafi, 2007).

Single cell culture: Single cell was taken with the help of sterilized inoculating wire loop from slimy yellowish bacterial colony developed around the infected samples and further streaked on nutrient agar plate in zigzag manner. After streaking, plates were incubated at 25-26^{o}C for 3 days.

Pathogenicity test/confirmation of pathogenic nature: All isolates were subjected to the pathogenecity test to confirm their pathogenic nature by injection infiltrations technique developed by Klemet (1963) and Klemet *et al*., (1964).

Inoculation of rice germplasm in glass house: Distilled water (5ml) poured in each culture plates of bacterial colonies were suspended and the concentration of inoculums was adjusted to 10^8 cfu/ml. The suspension of all isolates was bulked in plastic bucket and shaked for uniformity. The plants were sprayed with water to create humid conditions which is favorable for disease development. Inoculation was done by cutting 5 leaves, approximately 5cm from the tips of leaf of each line with scissor after dipping in inoculums suspensions. On the basis of diseased data these germplasm was categorized as resistant or susceptible using standard IRRI procedure.

Reaction	Lesion Length
Resistant	1-5 cm
Mod resistant	5-10 cm
Mod susceptible	10-15 m
Susceptible	> 15

After 15 days, diseased data were recorded to identify the degree of pathogenecity on 0-4 rating scale, Standard Evaluation System IRRI, (1988).

Results and Discussion

Wild relatives are the reservoir of useful genetic variability for biotic and abiotic stresses. No information was available for evaluating wild relatives against *Xoo* from Pakistan. It is the 1st attempt to test wild rice against *Xoo* prevailing in Pakistan. Sixty diseased rice samples used for isolation of *Xoo*, 25 isolates were pathogenic. The interspecific crosses showed resistant to moderately susceptible reaction on inoculation with *Xoo.* The lesion developed on KS282 x *O. nivara*, was recorded with mean leaf lesion length 1.5cm exhibiting its resistant reaction. KS282 x *O. rufipogon* showed reaction giving mean leaf lesion length, 5.75cm which indicates its moderate resistant character. Bas.385 x *O. nivara* gave mean leaf lesion length of **1.5cm** showing its **resistant** character. The cross involving IR6 and *O. nivara* gave mean leaf lesion length 1cm while IR6 x *O. rufipogon* giving mean leaf lesion length of 5.5 cm indicating resistant and moderately resistant reaction respectively (Table 1).

Table 1. Mean leaf lesion length of interspecific crosses of rice.

S. No	Variety/Line	Mean lesion length
1.	KS282 x *O.nivara*	1.5 ± 0.8
2.	Bas. 385 x *O.nivara*	1.5 ± 0.7
3.	IR6 x *O.nivara*	1.0 ± 0.4
4.	Bas.385 x *O.rufipogon*	13.0 ±0.6
5.	KS282 x *O.rufipogon*	5.75 ±0.7
6.	IR6 x *O.rufipogon*	5.5 ±0.8

Eight wild species of rice used individually in order to check its resistance level. *O. grandiglumis* gave mean leaf lesion length 4.8cm indicating resistant character. *O. rufipogon* exhibited mean leaf lesion length 12 cm showing moderately susceptible character while *O. austaraliensis, O. offcinalis, O. nivara, O. latifolia, O. longistminata* and *O. punctata* showed resistant character (Table 2). All new plant type lines gave mean leaf lesion length of more than 15 cm which clearly indicates its susceptible character (Table 3).

Table 2. Wild rice showing mean leaf lesion length.

S. No.	Variety/Line	Mean lesion length (cm)
1.	*O.grandiglumis*	4.8 ± 0.5
2.	*O.australiensis*	3.0 ± 0.8
3.	*O.officinalis*	3.5 ± 0.9
4.	*O.rufipogon*	12 ± 1.0
5.	*O.nivara*	1.0 ± 0.3
6.	*O.latifolia*	4.6 ± 0.6
7.	*O.longistminata*	1.0 ± 0.8
8.	*O.punctata*	1.6 ± 0.8

Table 3. Mean leaf lesion length of New Plant Type.

S. No	Variety/Line	Mean lesion length (cm)
1.	NPT line 73	4.8 ± 0.9
2.	NPT line 181	3.0 ± 0.9
3.	NPT line 191	All dry
4.	NPT line 18	12 ± 0.4
5.	NPT line 195	1.0 ± 0.8
6.	NPT line 163	4.6 ± 0.9
7.	NPT line 40	1.0 ± 2.0

Two commercial varieties and one mutant screened against *Xoo* in order to check its status of resistance to these varieties to current virulence of *Xoo.* Both commercial varieties, Bas.385 and KS282 showed susceptible character giving mean leaf lesion length of 16.8 cm and 18.4 cm respectively (**Table 4)** while one d1 mutant line gave mean leaf lesion of 6.6cm indicating its moderate resistant character (Table **5**). The near isogenic lines, IRBB24, IRBB94 gave mean leaf lesion length of 12cm and 5 cm respectively showing its resistant character. While rest of the lines, were moderately resistant to susceptible (Table 6).

Table 4. Mean leaf lesion length of commercial rice varieties.

S. No	Variety/Line	Mean lesion length (cm)
1	Bas.385	16.8 ±1.0
2	KS282	18.4 ±0.9

Table 5. Mutant type showing mean leaf lesion length.

S. No	Variety/Line	Mean lesion length (cm)
1	Kinmaze d1	6.6 ±0.7

Table. 6. Mean leaf lesion length of isogenic lines.

S. No	Variety/line	Mean lesion length (cm)
1	IRBB 05	15.8 ± 0.6
2	IRBB 25	09.6 ± 0.8
3	IRBB 09	06.2 ± 0.9
4	IRBB 07	13.6 ± 0.6
5	IRBB 50	18.8 ± 0.5
6	IRBB 14	06.2 ± 0.6
7	IRBB 08	09.8 ± 0.5
8	IRBB 59	12.8± 0.2
9	IRBB 94	05.0 ± 0.6
10	IRBB 57	19.2 ± 0.6
11	IRBB 04	12.4 ± 0.7
12	IRBB 11	11.8 ± 0.8
13	IRBB 52	12.3± 0.9
14	IRBB 54	10.8 ± 0.9
15	IRBB 60	10.6 ± 0.6
16	IRBB 58	18.8 ± 0.5
17	IRBB 10	12.8 ± 0.8
17	IRBB 55	18.8 ± 0.7
18	IRBB 01	06.0 ± 1.0
19	IRBB 21	16.6 ± 0.6
20	IRBB 08	29.8 ± 0.8
21	IRBB 24	01.2 ± 0.5
22	IRBB 53	09.6 ± 0.9

Oryza nivara was evaluated against *Xoo* and showed resistant character. Similar results were also reported by Kaur *et al*., (2005) using *Xoo* from india. Khush *et al*., 1990 reported new genes for resistance to BB from *O. longistminata*. This gene confers resistance to all Philippines race of BB was *Xa21*. He showed that its level of resistance is high and is distinct from entire known gene identified so far. Several reports showed that commercial cultivars of rice from Pakistan are susceptible to BB. Shah (2006) evaluated Bas.385 and KS282 against *Xoo* and he showed both varieties produced maximum lesion length against *Xoo* which clearly showed its

susceptible character. Khan *et al*., (2000) screened Bas.385 and KS282 under field condition and found that both varieties were susceptible to BB. Cheema *et al*., (1998) also tested Bas.385 against BB and showed that this variety failed to resist this pathogen.

So far 22 major genes for resistance to BB have been identified (Khush & Angels, 1999; Kihupi *et al*., 2001) and several of these genes have been incorporated into improved commercial varieties which are now widely grown in the world. As new races of the pathogen are developing continuously, hence there is a need to identify new sources of resistance. In the present study, new sources of resistance against *Xoo* were identified. *O. nivara*, *O. longistminata*, and *O. punctata* are recommended for utilizing in rice breeding program.

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(Received for publication 29 May 2009)