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VIRULENCE / AGGRESSIVENESS TESTING OF *XANTHOMONAS ORYZAE* PV. *ORYZA* ISOLATES CAUSE OF BLB DISEASE IN RICE CULTIVARS OF PAKISTAN

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Abstract

In present study, virulence and aggressiveness of seven *Xanthomonas oryzae* hypersensitive isolates, (Xoo 20, Xoo 36, Xoo 51, Xoo 65, Xoo 74, Xoo 99 and Xoo 105) were tested on seven rice varieties (Basmati 385, IRRI 26, Basmati 386, Dilroosh 97, JP 5, Super Basmati, Basmati 2000 and Ks 282), through detached leaf, glass house and field assays Xoo 99 showed most aggressive reaction on detached leaves and Xoo 105 exhibited more aggressive reaction on glass house plant and field assay as compared to other isolates. Moreover, all rice tested cultivars showed susceptibility against isolates of BLB disease, Basmati 385 were showed more susceptible reaction.

Introduction

Bacterial leaf blight (BLB) is the most destructive and very serious disease causing million of tones of grain losses annually specially in rice growing countries. It has been reported from all continents of the world, except Europe (Alim, 1967; Ou, 1985). Bacterial leaf blight was first time reported in Pakistan in 1977 (Mew & Majid, 1977. In Pakistan the incidence of BLB has increased in recent years especially in Kaller belt.

After studying the severity and significant damage caused by this destructive disease world wide, efforts are made to control BLB by using different methods, but no approach is considered as an effective safe, eco-friendly and economic (Devadath, 1970). The scientists focused their attention on its control and management by using resistant varieties against *Xanthomonas oryzae* pv. *Oryza.* Aim of present study is to assess virulence and aggressiveness of different isolates of *Xanthomonas oryzae* on varieties of rice, and it will be further used in breeding experiments, and its long term strategy to mange BLB disease of rice.

Materials and Methods

a. Detached leaf assay: To determine the virulence and relative aggressiveness, the suspensions of different isolates of *Xanthomans oryzae* (Xoo 20, Xoo 36, Xoo 51, Xoo 65, Xoo 74, Xoo 99 and Xoo 105) obtained from the four rice zones were applied on leaves of eight rice varieties (Basmati 385, IRRI 26, Basmati 386, Dilroosh 97, JP 5, Super Basmati, Basmati 2000 and Ks 282). The detached leaves were placed on 3-folded blotting paper towel in petri plates and inoculated with bacterial suspension containing 10^8 cfu/ml through pin prick method. The inoculated leaves were incubated at 22°C, the lesion length measured in cm and data analyzed statistically by ANOVA and significance at 5% level was tested by Duncan's multiple range test (DMRT)

b. Glass house assay: In glass house experiment seeds of eight rice varieties (Basmati 385, IRR1 6, Basmati 386, Dilroosh 97, Jp5, Super Basmati, Basmati 2000 and Ks 282) were grown on moist sterilized filter paper in Petri plates, maintained in a growth chamber at 30-35°C. Two weeks old seedlings were transplanted to small plastic pots (diameter 13 cm) and placed in glass house at pre-tillering stage. The plants were again transplanted to bigger plastic pots (diameter 27 cm) and three leaves per isolate were inoculated using clipping method. The control was treated simply with sterilized phosphate buffer saline (PBS). The lesion size was measured after 12-14 days.

c. Field assay: Field trials for testing pathogenicity/ virulence of different isolates of bacterium *Xanthomonas oryzae* Pv. *oryzae* were conducted at fields of NARC (National Agriculture Research Centre) Islamabad, Pakistan.

Field nursery: For nursery raising the seeds of rice variety Basmati 385 and Super Basmati were soaked (100g/m^2) overnight and sown during the first week of June. The seeds were spread on seed bed covered with dried plant material (wheat or rice straw) and kept moist by adding water. After one month (in the first week of July) the seedlings were removed from the nursery and transplanted in the field.

Preparation of bacterial inoculum: The cultures of the different isolate were prepared streaking a loop full of each isolate in the middle of nutrient agar plates and inoculated at 28°C. The bacterium was washed from plate surface after 24h with 5ml of saline distilled water (SDW). The inoculum was serially diluted and adjusted to a concentration of 10^8cfu ml^{-1}.

Inoculation/treatment: Sixty to seventy days old rice plants were inoculated with isolates of *Xanthomonas oryzae*, using clipping method of inoculation. The lesion size was measured after 12-14.

Result and Discussion

Seven isolates of *Xanthomonas oryzae* Pv.*oryzae* (Xoo 20, Xoo 36, Xoo 51, Xoo 65, Xoo 75, Xoo 99, Xoo 105) were examined for their aggressiveness. Through detached leaf assay, glass house and field assay. All isolates of *Xanthomonas oryzae* were pathogenic on all tested varieties of rice, but they were different in their aggressiveness behavior. Using two factorial T tests each isolates' reaction was compared individually on eight different cultivars in terms of mean lesion length. The isolates Xoo 51, Xoo 75, Xoo 99 and Xoo 105 showed maximum aggressive reaction against all test varieties of rice. However, at $p<0.05$, isolate Xoo 99 showed significant, highly aggressive reaction on rice variety Basmati 385 and produced large lesion size, 14.2 cm in length through detached leaf assay (Table 1; Fig. 1) The detached leaf assay was effective because of its reproducibility due to similar size

and age of leaves, effortless handling of leaves, uniform incubation period, controlled assay conditions, minimum chance of contamination and easy determination of potential susceptibility of plant host. Sing *et al.,* (2005) studied differential rice cultivars responses to *R. solani* by *In vitro* detached leaf inoculation method using 14 field isolates from Arkansas, the major rice growing state of the United States. Various other scientists have used similar methods (Parke *et al.,* 2002 Rhodes *et al.,* 2005; and Irwin *et al.,* 2006). While Xoo 105 showed strong aggressive reaction on rice variety Bas 386 produced large size lesion length 24.97 cm. in length through glass house assay.(Table 1; Fig. 2) In field assay Xoo 105 formed large lesion on rice variety Bas 385 forming large lesion size 26.98 cm in length. (Table 1; Fig. 3). This is in agreement with the observations of Ezuka & Horino (1974) in Japan who tested 14 isolates for their pathogenicity to three rice cultivars, Kinmaze, Kogyoku and Te-tep. The data showed that there was distinct "differential interaction". Watanabe, (1976) studied on the breeding of rice varieties resistant to bacterial leaf blight in Sirilanka and evolved the terms of vertical differences (virulence) and horizontal differences in pathogenecity (aggressiveness). Noda *et al*., (1989) tested selected cultivars for quantitative resistance to isolates of different aggressiveness .He found that the lesion length on each cultivar was caused by compatible isolates which increased the degree of aggressiveness of the isolates. Similar results are also reported by (Kauffman *et al.,* 1973; Akhtar *et al*., 2003).

All rice cultivars especially Basmati 385, IRRI 26, Bas 2000 showed susceptible reaction while to some extent two varieties of rice IRRI 26, Dilroosh 97 showed moderately susceptible reaction but none of the rice variety showed resistance against *Xanthmonas oryzae* assessed by resistance evaluating system as developed by Baw & Mew (1988).

Table 1. Measurement of bacterial leaf blight lesion length (cm) on different leaf assay of rice varieties.

Assays	Isolates	Rice varieties Bas 385	IRRI 6	BAS 386	Dilroosh 97	JP 5	KS 282	Super Bas	Bas 2000	Mean
Detached leaf assay	Xoo 20	8.33klmn	5.26r	8.46klmn	5.53qr	7.73lmno	7.8mno	8.43klmn	8.43klmn	7.47d
	Xoo 36	6.46pq	5.4qr	7.16hij	6.80p	7.63mnop	6.80p	6.930p	6.9nop	7.34d
	Xoo 51	12.8bcdefg	12.6defgh	13.63abcd	12.73cdefg	13.5abcdef	11.8efghi	12.66cdefgh	11.8ghi	12.67b
	Xoo 65	9.16k	9.2k	8.8klm	8.26klmn	13.5mno	8.5klm	8.46klm	9kl	8.66c
	Xoo 75	13.73abcd	12.9bcdefg	13.5abcd	11.9fghi	7.73abcd	13.96abc	13.13abcdef	13.63abcd	13.30a
	Xoo 99	14.2a	12.7cdefg	13.56abcd	12.36efghi	13.96abc	13.9abcd	13.2abcde	13.8abcd	13.42a
	Xoo 105	12.5abcd	10.46abcdefg	13abcdefg	11.26ij	12.83bcdefg	10.93ij	13.76abcd	14.1aa	12.47b
	Mean	**11.17b**	**9.79d**	**11.78a**	**9.79d**	**10.97b**	**10.54c**	**10.96b**	**11.11b**	
Potted plant assay	Xoo 20	8.8stuv	8.5tuv	9.36qrstu	7.5w	8.68tuv	9.23rstu	9.63pqrst	9.26rstu	8.85e
	Xoo 36	9.3rsto	9.73pqrs	11.43klm	8.5tuv	10opqr	9.26rstu	9.23rstu	9.56pqrst	9.63d
	Xoo 51	14.23gh	10.43mnop	14.7g	11.06lmno	11.5kl	10.76lmno	13.53hi	14.06gh	12.54c
	Xoo 65	9.56pqrst	7.76vw	10.5lmnop	8.4uvw	8.66tuv	8.5tuv	8.6tuv	8.8stuv	8.85e
	Xoo 75	22.1c	12.3jk	24.3ab	11.23lmn	22.76c	17.30f	22.76	22.73b	19.31b
	Xoo 99	22.83c	13.06ij	22.5c	10.5lmnop	24.6ab	17.6e	24.17ab	23.73b	19.88a
	Xoo 105	22.67c	10.33nopq	24.97a	11.43klm	24.7ab	18.5d	24.5ab	23.9b	20.13a
	Mean	**15.64c**	**10.30e**	**16.84a**	**9.8f**	**15.85bc**	**12.89d**	**16.04b**	**15.99b**	
Field assay	Xoo 20	20 .67	18.55	22.56	17.89	20.54	17.99	22.56	23	20.44
	Xoo 36	19.50	20.0	19.34	20.77	18.99	19.87	20.22	21.67	22.9
	Xoo 51	22.23	18.9	19.78	19.98	20.56	20.66	20.78	22.77	23.66
	Xoo 65	19.34	19.29	20.0	20.0	19.88	16.88	19.78	23.89	22.72
	Xoo 75	21.50	20.22	19.50	18.99	19.0	19.99	20.98	22.0	23.16
	Xoo 99	23.30	26.22	25.22	24.78	23.66	20.66	24.78	24.0	27.56
	Xoo 105	26.98	25.5	26.86	25.87	23.98	23.77	26.0	25.85	29.2
	Mean	**18.93**	**21.24**	**21.89**	**21.18**	**20.94**	**19.97**	**22.15**	**23.13**	

Fig. 1. Measurement of BLB lesion length (cm) through detached leaf assay.

Fig. 2. Measurement of BLB lesion length (cm) through Glass house assay.

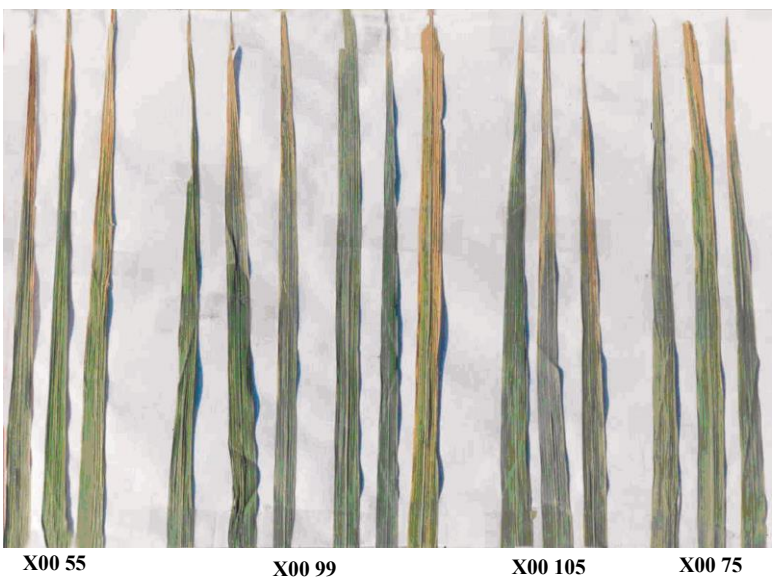

Fig. 3. Measurement of BLB lesion length (cm) through field assay of rice.

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