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EVALUATING BLB RESISTANCE / AGGRESSIVNESS IN RICE THROUGH BEST INOCULUM CONCENTRATION, INOCULATION AND APPLICATION METHODS

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Abstract

The present study was conducted to compare effective method of inoculation, inoculum concentration and application of agrochemicals for evaluating BLB resistance/ aggressiveness and management of BLB disease in rice susceptible varieties through detached leaf assay, Glass house assay and field assay against most aggressive isolate Xoo 105. A comparison of inoculation methods showed that pin prick method produced high infection in all cultivars than clipping and paint brush methods. The pin prick method significantly produced high lesion length on detached leaves as compared to other two methods, while clipping method of inoculation showed high infection producing large lesion in potted plant and field assays. Protective application methods of streptomycin drug showed best results as compare to curative and promising concentration of 10^8cfu /ml.

Introduction

Bacterial leaf blight (BLB) of rice is one of the most devastating and destructive disease of rice causing huge losses annually. It has been reported in all rice growing areas of the world including Pakistan (Ilyas & Javed, 1995; Khan *et al.*, 2000), except Europe. In Pakistan the incidence of BLB has increased in recent years especially in Kaller belt that is famous for producing high quality rice (Akhter *et al*., 2003). In view of the severity and significant damage caused by this destructive disease world wide, the scientists focused their attention on its control and management by using resistant varieties, chemical spray and bio-control agents.

The primary method of evaluating resistance, promising concentration of inoculum, inoculation methods and inoculum application is very essential because result may fluctuate with condition. Three methods, needle prick, spray, and immersion inoculation were used to produce Kresek symptoms artificially, while the clipping inoculation method was used for their genetic analysis of resistant cultivars Ogawa *et al*., (1990).

In management of BLB two methods of application of drug (streptomycin) were used in protective and curative manner. In protective method drug were used before inoculation of pathogen, while in curative it was applied after inoculation of pathogen. Protective method is found to be best (Aktar *et al*., 1997).

The objective of the present study was to evaluate best inoculation method, promising inoculum concentration and best application method for further study of pathogencity experiment and management of BLB disease effectively.

Material and Methods

Methods of inoculation

a. Detached leaf assay: Three methods of inoculation, pin prick, clipping and paint brush were compared through detached leaf assay. The fully expanded leaves of rice varieties Basmati 385, IRR1 6, Basmati 386, Dilroosh 97, Jp5, Super Basmati, Basmati 2000, Ks 282 were collected from glass house in plastic bags. These were washed with water, sterilized with 70% ethanol for 1 minute and rinsed twice with SDW. Three leaves were placed on four layers of sterile blotting paper towel in covered Petri plates. The leaves were inoculated with suspension of aggressive bacterial isolates serially diluted and adjusted to a concentration of 10^2cfu / ml^{-1}, 10^4cfu / ml^{-1}, 10^8cfu ml^{-1} using clipping method in which sterile scissors dipped in inoculum were used to cut 3 cm at leaves tip (Kauffman *et al*., 1973). In pin prick method sterile pins dipped in inoculum were used to prick the leaves on sides and in the center (Di *et al*., 1991). In paint brush method the leaves were painted with inoculum. Streptomycin drug 120mgl $^{-1}$ was used for testing application method. In control, the leaves were inoculated with sterile phosphate buffer saline (PBS). The leaves were incubated at 22^{o}C and the lesion length was measured in cm. The treatment was replicated thrice and data were analyzed statistically by analysis of variance (ANOVA) and significance at 5% level was tested by Duncan's multiple range test (DMRT).

b. Glass house assay: In glass house assay three methods of inoculation were compared. The eight varieties of rice Basmati 385, IRRI 26, Basmati 386, Dilroosh 97, JP 5, Super Basmati, Basmati 2000 and Ks 282 were grown on moist sterilized filter paper in Petri plates, maintained in growth chamber at 30–35^o C (100% Rh). Two weeks old seedlings at tillering stage were transplanted in small plastic pots (diameter 13 cm) and shifted to green house. After one week the plants were again transferred to bigger plastic pots (diameter 27 cm). Three leaves of each variety were inoculated with suspension of *Xanthomonas oryzae* isolate Xoo 105 The inoculum was serially diluted and adjusted to a concentration of 10^2cfu ml^{-1}, 10^4cfu / ml^{-1}, 10^8cfu /ml^{-1}.through clipping, pin prick and paint brush method respectively. streptomycin drug 120 mg l^{-1} used for testing application method The control leaves were inoculated with sterile phosphate buffer saline (PBS). Each treatment was replicated thrice. The plants were placed in glass room under natural conditions. The lesion length was measured in cm and data were analyzed statistically by ANOVA and significance at 5 % level was tested by Duncan's multiple range tests.

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c. Field trials: Field trials were conducted at fields of NARC (National Agriculture Research Centre) Islamabad, Pakistan.

i. 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.

ii. Preparation of bacterial inoculum: The cultures of the most aggressive isolate were prepared by streaking a loop full of each isolate in the middle of nutrient agar plates and inoculated at 28^oC. 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 10^2cfu / ml^{-1}, 10^4cfu/ ml^{-1}, 108cfu /ml^{-1}.

iii. Inoculation/treatment: Sixty to seventy days old rice plants were inoculated with most aggressive isolates of *Xanthomonas oryzae*, using clipping method of inoculation, the curative and protective methods of application of Streptomycin sulphate 120mgl $^{-1}$ was used as mentioned earlier.

Results and Discussion

Three methods of inoculation, clipping, pin prick and paint brush were tested both on detached leaves and on attached leaves *In -vitro* and *In- vivo* experiments. All three methods were effective for artificial inoculation, but pin prick method was found to be more efficient in detached leaf assay (Fig. 1) produced large size lesion (15.46 cm).

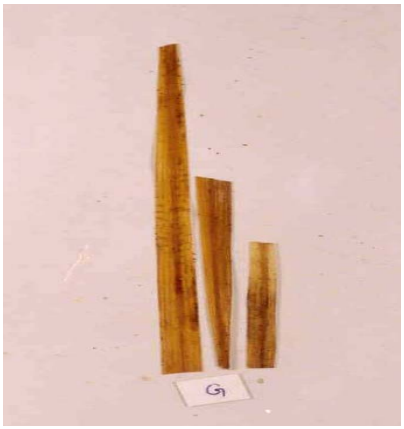

Fig. 1. Evaluation of pin prick method on detached leaf assay.

The pin prick method of inoculation proved most suitable for quantitative determination of causal bacterium. The cell sap coming from injured tissue due to pin pricking usually serves as nutrient for the bacteria. This causes bacterium to multiply and increase in hypertrophic region. After that the bacterium moves towards non-hypertrophies intercellular spaces. The method though is suitable for accurate evaluation but is laborious and time consuming for glass house and field assays (Table 1; Fig. 1).

Table 1. The Measurement of Bacterial Leaf Blight through best inoculation concentration, inoculation and application methods.

Assays	Bacterial blight lesion length (cm)/ Isolate XOO 105								
	Rice varieties	Inoculum concentration			Inoculation methods			Application/ streptomycin drug	
		10^2cfu /ml	10^4cfu /ml	10^8cfu /ml	Pin prick	Clipping	Paint Brush	Protective	Curative
Detached leaf assay	Bas 385	4.3r	6.46pq	12.8bcdefg	13.3AB	10.5D	9.7E	12.37jk	8.4n
	IRRI 6	5.26r	5.4qr	12.6defgh	9.03EF	13.13BC	3.3J	13.77i	10.67l
	KSh BAS 385	8.46klmn	7.16hij	13.63abcd	13.3AB	12.56C	5.46II	10.7l	5.46s
	Dilroosh 97	5.53qr	6.80p	12.73cdefg	12.8BC	10.46D	6.13H	13.7i	8.60n
	JP 5	7.73lmno	7.63mnop	13.5abcdef	12.8BC	10.93D	8.5F	11.07l	6.3qr
	KS 282	7.8mno	6.80p	11.8efghi	12.46C	10.6D	16.1H	12.60j	9.60m
	Super Bas	8.43klmn	6.930p	12.66cdefgh	15.46F	10.5D	6.46H	9.6m	4.03u
	Bas 2000	8.43klmn	6.9nop	11.8ghi	12.53C	10.73D	7.63G	14.07hi	4.9st
Glass house assay	Bas 385	8.6tuv	22.76	24.17ab	13.16H	18.13DE	7.16L	6.13r	12.20jk
	IRRI 6	7.76vw	12.3jk	13.06ij	11.46I	13.16H	5.46M	7.23op	13.50i
	KSh BAS 385	10.5lmnop	24.3ab	22.5c	18.5D	22.46B	7.16L	6.68l	12.85e
	Dilroosh 97	10opqr	11.5kl	18.00F	15.8F	14.6G	3.53N	6.13r	12.20jk
	JP 5	8.66tuv	22.76c	24.6ab	21C	24.96A	7.46N	7.23op	13.50i
	KS 282	8.5tuv	17.30f	17.6e	17.5E	24.46A	9.23K	6.68l	12.85e
	Super Bas	8.6tuv	22.76	24.17ab	15.96F	24A	10.56J	6.76pq	12.7j
	Bas 2000	8.8stuv	22.73b	23.73b	16.5F	24A	10.8IJ	7.50o	11.97k
Field assay	Bas 385	20.66	24.50	27.00	15.5	17.67	18.56	14.90fg	21.80a
	IRRI 6	27.43	28.00	28.23	18.55	2.67	18.98	15.83e	21.83ab
	KSh BAS 385	21.50	23.80	25.60	17.98	25.45	17.66	14.77g	22.17a
	Dilroosh 97	18.53	24.83	25.33	18.56	26.56	20	14.40gh	21.90ab
	JP 5	13.80	22.90	28.23	18.00	24.44	23.22	13.53i	22.0ab
	KS 282	20.22	24.17	27.90	19.70	26.5	18.98	15.90e	21.87
	Super Bas	16.98	23.54	24.56	18.99	24.79	18.58	18.07d	21.97ab
	Bas 2000	12.54	23.72	26.99	19.65	23.98	20.47	18.80c	21.93ab

The clipping method of inoculation was found more effective in glass house assay producing large lesion size (24.96cm) and in field assay produced lesion size (24.79 cm). The clipping method of inoculation was found to be more quick and efficient in glass house and field assay. It was also observed at International Rice Research Institute (IRRI) by Kauffman *et al*., (1973).The leaves of each plant are grasped and the top of the leaves are clipped by a pair of scissor dipped in bacterial suspension. The cut ends provide entry for pathogen and might be one of the main causes for spreading BLB disease in rice plant (Table 1; Figs. 2, 3).

The method was reported to be useful not only for evaluating qualitative resistance but also quantitative resistance (Kaku *et al*., 1980). Highly significant correlation was found between clip and needle prick, clip and spray and clip, cut and spray inoculation methods (Horino *et al*., 1981). The clipping inoculation method for their genetic studies was found most efficient for genetic analysis of resistance cultivars, by Ogawa *et al*., (1990). In our case the paint brush method of inoculation was found to be less effective as compared to other two methods but it is more similar to natural infection. Mew *et al*., (1979) compared to the needle prick, clip and spray technique and found that neither lesion length nor disease score were affected by inoculation method, although the incubation period was longer in spray inoculated plants than in other plants.

Fig. 2. Evaluation of clipping method on 60-70 days old potted rice plants.

Fig. 3. Evaluation of clipping method on 60-70 days old field rice plants.

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