Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 7, No. 3, p. 59-65, 2015
http://www.innspub.net

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RESEARCH PAPER

OPEN ACCESS

Response of rice (*Oryza sativa* L.) germplasm against *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) under greenhouse conditions in Pakistan

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Article published on September 17, 2015

Key words: Rice, Bacterial leaf blight, *Xanthomonas oryzae* pv. oryzae, Screening, Germplasm.

Abstract

Bacterial leaf blight (BLB) is one of the apex devastating diseases of the rice crop around the world. It continuously reduced the yield and quality of rice. In this investigation efforts have been made to study the response of different rice germplasm against *Xanthomonas. oryzae* pv. *oryzae (Xoo)* under greenhouse conditions. Diseased leaves were collected from the major rice growing areas of Punjab during 2011. Thirty five *Xoo* isolates were purified and confirmed. For pathogenicity test Super basmati rice variety was used. Plants were inoculated with freshly prepared inoculum (10^9 CFU/ml) of PKXOO4 (an aggressive strain) by leaf clip method. Among 50 lines screened, one was found highly resistant, one was resistant and two were moderately resistant against *Xoo*. The resistant germplasm identified can be used in breeding rice varieties against *Xoo*.

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Introduction

In Pakistan, rice is an important food and cash crop, the second staple food crop after wheat and is second major exportable commodity after cotton. It is planted more or less on an area of 2.5million hectares and annual production of 5.5million tones. It accounts for 4.4 percent of value added in agriculture and 0.9% in GDP (Economic Survey of Pakistan, 2013-14).

Rice, an important food and cash crop, throughout the world including Pakistan but it is attacked by a number of diseases. These pathogens attack rice crop at all stages and lead to qualitative and quantitative losses. Our national yield is low as compared to some other rice growing countries likes USA (7.0tons/ha), Japan (6.7tons/ha), China (6.2tons/ha) and Indonesia (4.4 tons/ha). At the same time the yield of aroma basmati rice is less than other modern varieties. Our national yield of scented rice is around 1.8 tons/ha. Asghar *et al.*, 2007 reported that the damage from rice diseases is enough to feed 60 million peoples and cause losses of 5 billion dollars.

The disease is caused by a gram negative bacteria called *Xanthomonas oryzae* pv. *oryzae* (Swing *et al.*, 1990) and is one of the most destructive diseases in all rice growing areas of the world. In Asia, BLB has the status of major disease and most of the rice cultivars are susceptible against it.

In Pakistan the disease was first reported in Kallar belt of Punjab in 1977 and later its occurrences was confirmed from all over the country (Akhtar and Akram, 1987). In Pakistan symptoms of disease usually appear at tillering stage. The disease incidence increases with plant growth and is on its peak at flowering and grain formation stage. Yield reduction under mild BLB infection is 10-20% (Mew *et al.*, 1993) whereas in severely infected fields yield may be reduced up to 50% (Ou, 1985).

The disease is controlled by chemicals but due to lack of specific bactericides, the management of this disease is very difficult. The farmers are not well aware about bacterial leaf blight disease symptoms and they do not spray bactericides at proper time. Therefore, host plat resistance has emerged as the best option to control BLB (Gnanamanickam *et al.*, 1999; Tang *et al.*, 2000; Sheng *et al.*, 2005). Rice germplasm used in our country is susceptible against *Xanthomonas oryzae* pv *oryzae* isolates (Akhtar, 2005; Tasleem *et al.,* 2000).Therefore, It is very necessary to search the resistances sources against this disease. In this investigation efforts have been made to study the response of different rice germplasm against PKXOO4 (an aggressive strain) under the greenhouse conditions.

Materials and methods

Isolation of causal organism

The diseased leaves were collected from rice fields of Punjab during 2011 rice season. The leaves were cut into small pieces. The small pieces were placed in1% Clorox for one minute and then in 70% ethanol for one minute. Finally they were washed with autoclaved distilled water at least three times and placed on peptone sucrose agar (PSA) and modified Wakimoto's agar media in petri plates at 28C^{o} in an incubator (Wilson *et al.*,1987). The yellow bacterial colonies with circular entire margins, smooth and shiny surface were isolated.

Preservation of XOO

The purified isolates were stored in 20% glycerol at -80 C^{o} for further studies.

Pathogenicity test

Seeds of elite commercial cultivar Super Basmati were sown in wooden trays and after one month seedling were transplanted in 25 cm diameter plastic pots. Rice plants were grown under greenhouse conditions. For pathogenicity test, plants were inoculated with PKXOO4 (the most aggressive strain of *Xoo*). Inoculum was prepared in 5ml of sterile distilled water @10^{9} CFU/ml. Fully fresh and expanded leaves were inoculated by leaf clipping method (Kauffman *et al.,* 1993) before panicle initiation. BLB lesion on clipped leaves was observed 15 days after inoculation ((Standard evaluation system for rice, IRRI. 1996).

Inoculation of rice germplasm under greenhouse condition

Fifty rice (*Oryza sativa* L.), lines including 46 lines collected from IRRI, 3 unapproved varieties (these are grown in Punjab) and one susceptible check TN1, were obtained from rice program, NARC, Islamabad. The plants were raised and inoculated as described above. The following rating scale was used for recording the disease infection (Table 1). *Dendrogram* was constructed using disease lesion length% and were subjected to cluster analysis by Euclidean distance Statistica software 0.7.

Results and discussion

Disease management by resistant varieties is a key component of integrated pest management (IPM) which can be easily adopted against any disease especially in the case of BLB where disease control by bactericide is a difficult task.

Therefore in this study 50 rice lines including (local and exotic) were screened against *X. oryzae* pv. *oryzae* pathogen to find out the resistance source. Results of the present study revealed that 1 wild rice species *O. brachyantha* showed highly resistant response whereas 1 wild species *O. rufipogon* showed resistant response (Table 2).

Table 1. Standard evaluation system for rice, IRRI. 1996.

Infection %	Score	Response
0 -3 %	1	Highly Resistance(HR)
4 – 6 %	2	Resistance(R)
7 -12 %	3	Moderately resistance(MR)
13 - 25 %	4	Moderately resistance(MR)
26 - 50 %	5	Moderately susceptible(MS)
51 - 75 %	6	Susceptible(S)
76 - 87 %	7	Susceptible(S)
88 - 94 %	8	Highly susceptible(HS)
95 - 100 %	9	Highly susceptible(HS)

Two rice genotypes showed moderately resistant response (MR), 9 rice genotypes were moderately susceptible (MS), 33 rice genotypes exhibited susceptible response(S) whereas 4 rice genotypes showed highly susceptible response (HS). Based on this response rice germplasm were classified into two major groups. Each further divided into three sub groups. Group A (sub-group A1,sub group A2 and sub-group A3), group B (sub-group B1,sub-groupB2 and sub-group B3) Group A consisted of 4 rice germplasm , out of which 1 belonged to sub group A1, 2 fell into sub group A2 and 1 belonged to sub group A3. *O. brachyantha* with 1 % lesion length value showing highly resistant response (HR) fell in sub group A1. Similarly in sub group A2, the disease lesion length% age value of *O. rufipogon* was 3.53%, it fell in resistant (R) class whereas disease lesion value of IR65483-104-11-4-23-B was 10.43% and it fell into moderately resistant (MR).In Sub group A3, rice line IR65483-118-25-31-7-1-5-B with disease lesion length % value of 14.84% fell into moderately resistant (MR).

Group B consisted of 46 rice germplasm, out of which 4 belonged to sub group B1, 9 to subgroup B2 and 33 to sub group B3. Sub group B2 is further divided into B2a and B2b. Similarly sub group B3 is also sub divided into B3a and B3b. In group B1, the disease lesion length% age values of TN1 was 94.91%, TKM6 (92.52%), IR03W127 (91.42%) and IR07W104 (90.98%). The entire group fell into highly susceptible (HS) group. Similarly In group B2a, the disease lesion length% values were as followed SHETE (36.04%), IR65483-104-13-13-22-1-B (36.90%), IR02w104 (36.96%) and IR56 (32.44%). In sub group B2b, the disease lesion length % value of IR02W110, IR65483-113-5-2-18-B, Kashiung Sen Yu, IR65483-106-8-2-19-B and IR03W132 were 47.56%, 45.55%, 43.03%, 43.44% and 43.75% respectively. These two sub groups showed moderately susceptible (MS) response. Sub group B3 comprised of 33 rice lines, out of which 33 belonged to sub group B3a and 2 belonged to sub group B3b. According to disease lesion length%, this group fell into susceptible (S) rice genotypes.

Table 2. Reaction of rice germplasm against *Xanthomonas oryzae* pv. *Oryzae*.

Entry no	Designation	Disease %	Scale	Response
1	IR64	52.94	6	S
2	IR07W101	60.58	6	S
3	IR07W102	68.36	6	S
4	IR73382-85-9-1-2-3-B-1-B	63.93	6	S
5	IR07W105	79.31	7	S
6	IR07W104	90.99	8	HS
7	IR00W101	71.65	6	S
8	IR06W102	70.24	6	S
9	IR73382-7-12-1-4	64.57	6	S
10	IR73680-3-1-14-1-4	67.62	6	S
11	IR55423-01	71.26	6	S
12	IR03W132	43.75	5	MS
13	IR03W133	57.45	6	S
14	IR02W110	47.56	5	MS
15	IR02W112	59.37	6	S
16	IR02W113	67.76	6	S
17	IR69502-6-SRN-3-UBN-1-B	67.84	6	S
18	IR03W134	65.93	6	S
19	IR03W127	91.42	8	HS
20	IR75870-8-1-2-B-6-1-1-B	81.81	7	S
21	IR80340-23-B-13-1-B-B	52.21	6	S
22	IR80351-25-B-27-B	76.19	7	S
23	IR56	32.44	5	MS
24	IR02W104	36.96	5	MS
25	IR65483-118-25-31-7-1-5-B	14.81	4	MR
26	IR65483-104-13-13-22-1-B	36.90	5	MS
27	IR02W107	69.69	6	S
28	IR65483-104-11-4-23-B	10.43	3	MR
29	IR65483-106-8-2-19-B	43.44	5	MS
30	IR65483-113-5-2-18-B	45.55	5	MS
31	IR65483-118-5-7-14-13-B	66.85	6	S
32	IR65483-6-4-21-B-B-1	73.97	6	S
33	IR65483-6-31-5-18-1	74.75	6	S
34	IR36	66.35	6	S
35	BIR ME FEN	71.96	6	S
36	TKM6	92.52	8	HS
37	GEB24	64.65	6	S
38	CO7	64.40	6	S
39	ARC 10982	72.22	6	S
40	CO36	84.74	7	S
41	SHETE	36.04	5	MS
42	KAOSHIUNG SEN YU 169	43.03	5	MS
43	PTB33	61.53	6	S
44	*O.rufipogon*	3.53	2	R
45	*O.brachyantha*	1.00	1	HR
46	SUPRI	80.00	7	S
47	Qayinat	81.76	7	S
48	PK.386	79.00	7	S
49	BAS.515	85.40	7	S
50	TN 1 (Control)	94.91	8	HS

Farmer usually applies fungicides/bactericides to control BLB disease in rice crop.

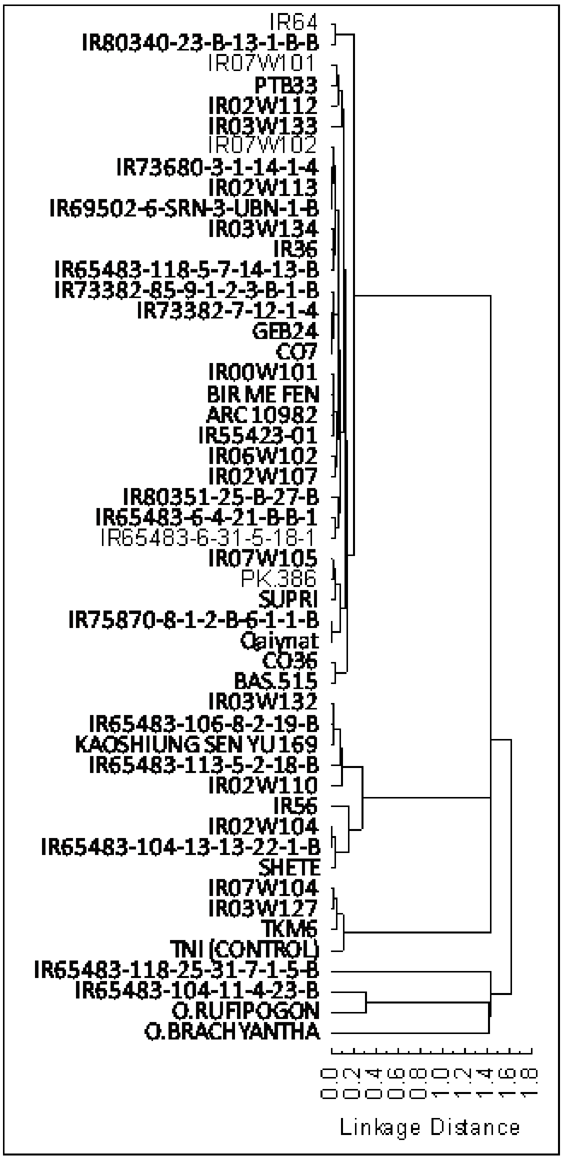

Fig. 1. Dendrogram of rice germplasm based on the disease lesion length%.

Misuse of pesticides is usually observed in rice fields. Pesticides related problems, like degradation of environment, health hazards to humans and animals, destruction of bio- diversity and contamination of food or feed etc. have often been reported. Alternate control measures either are not available or economically not feasible, efficient or farmers are not aware of them. The varietal resistance is considered as a key tool under any environment conditions to control insect pests or diseases in any type of crop. This method is more economical and convenient as compared to any other control method. It is also compatible with other control tactics of IPM.

Our results revealed that rice wild species *O. brachyantha* and *O. rufipogon* were highly resistant and resistant respectively. Wild rice species *O. brachyantha* and *O. rufipogon* are known to have resistance against *X. oryzae* pv. *oryzae* (Zhang *et al.*, 1994; Shah *et al.*, 2009; Akhter *et al.*, 2011; Kumar *et al.*, 2013). Further our results showed that IR64 and IR36 were susceptible against *Xoo*. These results are supported by the studies of Mondal *et al.*, 2012 whereas Hittalmani *et al.*, 2013 found IR64 and IR36 to be resistant against *Xoo*..TKM6 gave a highly susceptible response in our study whereas according to Thimmegowda *et al.*, 201.1 it showed resistant response against *Xoo*. Our results also indicated that PTB 33 is a susceptible variety; the results are different from those of Pophaly *et al.*, 1997 who reported this variety as resistant against BLB.

Two of the varieties PK.386 and Qayinat showed susceptible reaction against *X. oryzae* pv. *oryzae* which is a great concern as both the varieties are currently being grown by the farmers at a reasonably large area. Both of these varieties were also reported susceptible against *Xoo* previously (Khan *et al*, 2011; Jabeen *et al.*, 2012).

Bacterial diseases are among the major factors which limits crop productivity and are of great economic important for many crops, with the highest losses causes in cereals crops, vegetables and fruits (Morgues *et al.*, 2001). Control measures for bacterial leaf blight include cultural practice, chemical control, biological control and most important host genetic resistance. Since the other control measures are not effective, the utilization of resistant varieties carrying resistant genes have been considered to be the most effective way to control BLB (Nino-lu *et al.*, 2006). Thirty five BLB resistance genes have been identified in cultivated rice and wild relatives (Nino-lu *et al.*, 2006; Singh *et al.*, 2007; Wang *et al.*, 2009). Breeders have showed interest in utilization of BLB resistant germplasm and this goal is certainly achievable providing the availability of an easy strategy to identify resistance genes.

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