INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 7, No. 6, p. 103-110, 2015

RESEARCH PAPER OPEN ACCESS

Resistance Characterization of Cultivated Varieties and Rice Wild Species in Response to Bacterial Blight

Abdul Waheed*[1], Habib Ahmad[1], Fida. M. Abbasi[1], Azhar H. Shah[2], Hamid Ali[1], F. S. Hamid[3], Saqib Mumtaz[4]

[1]*Department of Genetics, Hazara University, Mansehra, Pakistan*

[2]*Department of Botany, Hazara University, Mansehra, Pakistan*

[3]*National Tea and High Value Crops Research Institute Shinkiari, Mansehra, Pakistan*

[4]*COMSATS Institute of Information Technology, Park Road, Islamabad, Pakistan*

Key words: *Oryza sativa*, Bacterial blight, Resistance, *Xoo* isolates (*Xanthomonas oryzae* pv *oryzae)*

http://dx.doi.org/10.12692/ijb/7.6.103-110 Article published on December 24, 2015

Abstract

Bacterial leaf blight (BB) of rice caused by (*Xanthomonas oryzae* pv *oryzae)* is converting into a critical threat almost in all rice growing countries of the world. In order to catagorize resistant sources to virulent isolates of BB, an experiment comprising 02 species of wild rice (*Oryza* sp.) and four most common cultivated varieties i.e., Bas-385 , Swat-1 , JP-5 and Fakhar Malakand of rice in Pakistan was conducted in the green house of Genetic Department Garden campus ,Hazara University in the rice growing season during 2012. Bacterial suspension of concentration 10^8 CFU/ml was prepared from mixture of (*Xanthomonas oryzae* pv *oryzae)* prevailing in Khyber Puktunkhawa,Pakistan i.e., *Xoo*-1, *Xoo*-2 and *Xoo*-3. Clip method of artifical inoculation was used. Both tested wild relatives of rice *O. longistaminata* and *O. rufipogon* showed highly resistance to all the isolate. F_3 genotypes Bas-385 x *O. rufipogon* was found highly susceptible to most of the isolates among all others genotypes. The use of resistant wild species *O. rufipogon* is therefore recommended in rice breeding program for transfer of bacterial blight resistant genes to cultivated varieties to enhance the relative characters.

* **Corresponding Author:** Abdul Waheed ✉ abdulw900@gmail.com

Introduction

Rice (*Oryza sativa* L.*)* is known as a staple food for more than half of the world's population (Chakravarthi & Naravaneni, 2006). Pakistan is an important rice growing and exporting country. Pakistani Basmati rice is famous for long grain aromatic character all over the world. International Rice Research Institute reported that, export share of Pakistani rice was 10 % of the total world rice trade (IRRI, 1993).

Many diseases of rice crop significantly reduce the yield and quality all over the world, among them the bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Akhtar, 2005) is the most destructive and critical disease of rice throughout the world (Mew, 1987). This disease was first observed by farmers in Japan during 1884-85 and its occurrence has been reported in Australia, Bangladesh, India, Mainland China, Malaysia, SriLanka, Thailand, Philippines, USA, West Africa and Vietnam (Ezuka & Kaku, 2000). Mew & Majid, 1977) reported its incidence in Pakistan and it was confirmed from all the provinces in a later study (Akhtar & Akram, 1987). Recently an alarming increase in BB incidence is observed in Pakistan especially in Punjab which is largest growing province of Pakistan and famous for rice cultivation (Khan *et al.*, 2000 & Akhtar *et al.*, 2003). Bacterial blight appears at all growth stages of rice and is manifested by either leaf blight or "Kresek" symptoms. The causal organism invades plants through water pores and wounds (Tabei & Mukoo, 1960). Since the water pores are located at the margins of upper parts of the leaf, the lesion starts from the leaf margins near its tip. As the disease progresses, the tiny water soaked lesions turns yellow, enlarges in size progressively and develop into an elongated irregular lesion with wavy margins. Bacterial ooze, which consists of small, yellowish, spherical masses, may sometimes be seen on the margins or veins of the freshly infected leaf under moist conditions. with the passage of time, the lesion may cover the entire blade, which turns white and later greyish owing saprophytic growth (Ou, 1985).

If plant ever produces panicles, it results in sterile immature grains, which are easily broken during milling. The reduction in yield in case of severe infection could be as high as 50% (Mew *et al.*, 1993) whereas 10-12% yield reduction has been recorded in case of mild infection (Ou, 1985). The disease is also characterized by a systemic infection phase, which is manifested by acute wilting of young plants. This is commonly referred to as "Kresek" phase. The causal organism consists of straight rods, with a single polar flagellum, occurring singularly, in pairs and sometimes in chains as well and is also Gram-negative (Swings *et al.*, 1990). The bacterium over winters either in weeds or in soil. Grains, straw and rice stubble are other possible sites of over wintering of the pathogen. During growing season, it enters the plants via natural opening or wounds where it survives and multiplies in plant's vascular system, producing typical leaf blight symptoms.

Bacterial bight has the potential to become a destructive disease of rice in Pakistan. Generally, the use of resistant cultivars is the most effective method for controlling plant diseases. However, the available rice germplasm in the country is susceptible to virulent isolates of bacterial blight (Akhtar, 2005). Rice productivity is limited by several biotic and abiotic stresses. Thus, there is an urgent need to wide extent the gene pool of cultivated rice. Rice wild species are an important source of variability for resistance to all major diseases, insects and pests, offered an important source of innovative resistance genes for rice crop improvement (Eizenga *et al.,* 2009). Wide hybridization between *Oryza sativa* (AA genome) and wild species of rice is one of the important way to transfer genes to cultivated rice. Apart from other research innovation some useful important genes have successfully been transferred from wild species of rice into cultivated rice to date which include genes for resistance to grassy stunt virus, bacterial blight, brown plant hopper, blast (Brar and Khush, 1997). The present study was, therefore, aimed to identify sources of resistant genes to virulent isolates of bacterial blight in wild relatives for future use in rice breeding programs.

Materials and Methods

The experiment was carried out in the green house of Genetic Department, Hazara University Garden Campus Mansehra during 2012 rice crop growing season.

Plant materials

Germplasm of 02 wild species of rice provided by International Rice Gene bank Collection, International Rice Research Institute (IRRI), Philippines along with commercial cultivars of rice in Pakistan viz. Bas-385, Swat-1, F. Malakand and JP-5, was used. The list of wild rice species along with their IRGC accession number and source is given in Table 1.

Establishment

Before seeding, seeds of wild relatives of rice were heat treated at 50^{O}C to break their dormancy. Seeding was done on sterilized Petri dishes. Seven days after seeding, seedlings were transplanted into well puddled pots of both length and diameter of 25 cm. To increase the number of plants, cloning of wild relatives was done. Clones derived from the newly arising tillers of existing plants were subsequently transferred into well puddled pots. Each clone later developed into full fledge plant.

Adult plants (100 days life) of wild relatives of rice representing different genomes along with commercial rice cultivars were inoculated with virulent isolates of *Xanthomonas oryzae* pv. *oryzae* prevailing in (Khyber PukhtunKawa) Province Pakistan i.e., Xoo-1, Xoo-2, and Xoo-3.

Pathogens

Isolates Xoo-1, Xoo-2, and Xoo-3 were collected from different rice growing areas of Punjab. Inoculum was prepared in distilled water. The concentration was adjusted to about 10^8 cfu/ml. The pairs of inoculating scissors were dipped into prepared inoculum placed in flasks wrapped with aluminum foil to protect bacteria from solar heat.

For each isolate, two leaves of each wild relative and commercial cultivar were cut at approximately 5cm from the tips and then lesion lengths were measured fifteen days after inoculation. On the basis of mean lesion size for each isolate, these wild relatives along with commercial cultivars were grouped into different categories of resistance and susceptibility using standard IRRI procedure (Chaudry, 1966).

Table 1. International Rice Gene bank Collection (IRGC) accession number and source countries of wild rice species (*Oryza* sp.) used in the study.

Wild rice sp.	IRGC accession	Source country
O. longistaminata	101200	Nigeria
O. rufipogon	103308	Taiwan

Score chart for evaluation response of host plant (Chaudry, 1996).

Lesion size (% of leaf length)	Disease rating scale	Category
0-3%	1	Highly resistant (HR)
4-12%	3	Resistant (R)
12-25 %	4	Moderately resistant (MR)
25-50 %	5	Moderately susceptible (MS)
51-87%	7	Susceptible (S)
87-100 %	9	Highly susceptible (HS)

Results

Three local isolates of *Xanthomonas oryzae pv oryzae* were provided by Crop Diseases Research Program (CDRP), National Agriculture Research Centre (NARC), Islamabad Pakistan. The isolates were preserved in dried form, in small vials.

To proceed further the strain were first revived on solid YDC (Yeast extract, Dextrose and Calcium carbonate), media.

Total 10 ml sterilized disttiled water added into each tube for revival . Thereafter, a wire loop full of bacteria were streeked-on petri plates with soild YDC media. These plates were incubated at 29^{0}C for 3 days.

Yellow, smooth colonies with round extermities appeared on petri plates (Figure 1). Fresh inoculum was prepared by pouring 15 ml sterile distilled water in each patri plate and leaf clip methhod was applied for inoculation of genotypes. The genotypes incubated at highest stage of tillering. After 14 days of inoculation data were recorded (Figure 2 and Table 3).

Table 2. Leaf length of cultivated varieties and wild species of rice inoculated with different *Xoo* isolates from PCoA.

Varieties/Isolates	***Xoo*-1**	***Xoo*-2**	***Xoo*-3**
	Leaf length (cm)	**Leaf length (cm)**	**Leaf length (cm)**
O. longistaminata	26	25	26
O. rufipogon	23	24	20
Bas-385	29	28	25
Swat-1	23	25	24
JP-5	19	21	21
Fakhre Malakand	18	21	20

Table 3. Mean leaf lesion length of cultivated varieties and wild species of rice inoculated with different *Xoo* isolates

Varieties/Isolates	***Xoo*-1**	***Xoo*-2**	***Xoo*-3**
	Lesion length (cm)	**Lesion length (cm)**	**Lesion length (cm)**
O. longistaminata	0.5	0.8	4.2
O. rufipogon	0.5	0.2	0.6
Bas-385	6.7	10.6	9.0
Swat-1	8.3	21.8	21.4
JP-5	3.4	2.7	6.1
FakhreMalakand	2.3	3.9	5.8

Oryza longistaminata showed mean leaf leision length of 0.5, 0.8 and 4.2 cm, on inoculation with *Xoo*-1, *Xoo*-2 and *Xoo*-3, respectively (Table 3). These lesions constituted 2, 3 and 16% of total leaf lengths, respectively. *Oryza longistaminata* was therefore highly resistant to *Xoo*-1 and *Xoo*-2 while moderately resistant to *Xoo*-3 (Table 4-5).

O. rufipogon manifested mean leaf lesions of 0.5, 0.2 and 0.6 cm, on inoculation with *Xoo*-1, *Xoo*-2 and *Xoo*-3, respectively. These lesions corresponded to 2, 1 and 3% of total leaf lengths, respectively. *Oryza rufipogon* was therefore highly resistant to all the three isolates of *Xoo* used in this study (Table 5).

Cultivated variety Bas-385 showed mean leaf lesions of 6.7, 10.6 and 9 cm, in response to *Xoo*-1, *Xoo*-2 and *Xoo*-3, respectively. These lesions were 23, 38 and 36 % of total leaf lengths. Consequently Bas-385 showed moderately resistant reactions to *Xoo*-I while moderately susceptible reactions to *Xoo*-2 and *Xoo*-3. Mean leaf lesions lengths recorded for JP-5 against *Xoo*-1, *Xoo*-2 and *Xoo*-3 were 3.4, 2.7 and 6.1 cm, respectively.

Fig. 1. Pure culture of *Xanthomonas oryzae pv oryzae* local isolates *Xoo*-3 grown on nutrient agar media.

That comprised infected area of 18, 13 and 29 %, respectively. JP-5 was thus, moderately resistant to *Xoo*-1 and *Xoo*-2. However, it was moderately susceptible to *Xoo*-3.

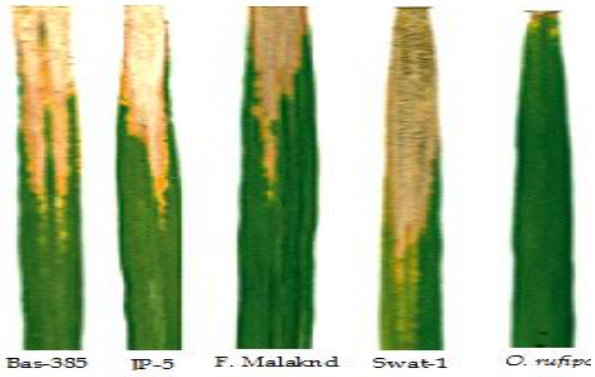

Fig. 2. Reactions of wild spp; and cultivated varieties of rice in response to *Xanthomonas Oryzae* pv *Oryzae* virulent strain *Xoo*-3.

Fakhre Malakand manifested mean leaf lesion length of 2.3, 3.9 and 5.8 cm, on inoculation with *Xoo*-1, *Xoo*-2 and *Xoo*-3, respectively. These lesions corresponded to 13, 19 and 28 % of total length of inoculated leaf.

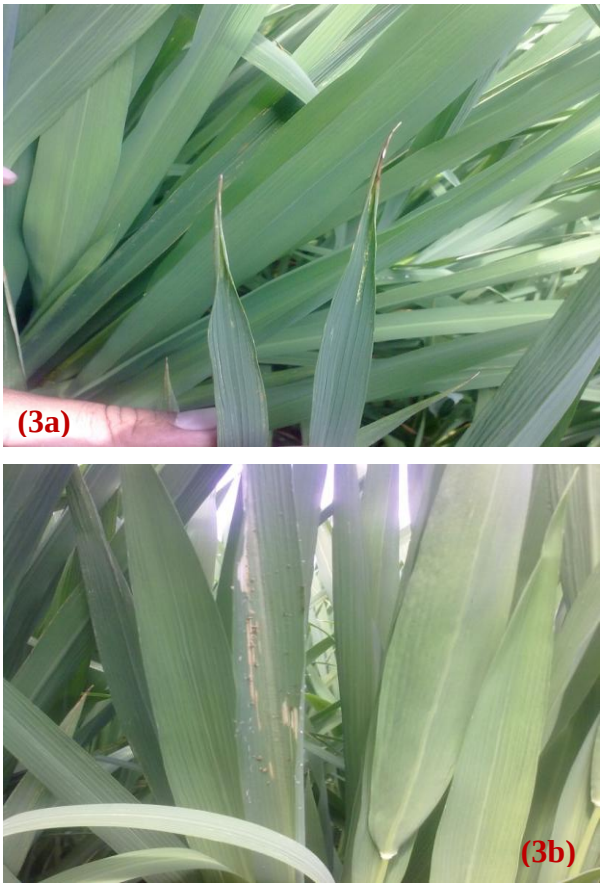

Fig. 3a, 3b. Isolates/strains *Xanthomonas oryzae* pv *oryzae* on *O. rufipogon* in green house

Fakhre Malakand was therefore, somewhat resistant to *Xoo*-1 and *Xoo*-2 where as somewhat susceptible to *Xoo*-3.

Swat-1 showed lesions of 8.3, 21.8 and 21.4 cm, to *Xoo*-1, *Xoo*-2 and *Xoo*-3, respectively. That corresponded to 36, 87 and 89 %, respectively. Therefore Swat-1 showed the most susceptible reactions in this study. It was moderately susceptible to *Xoo*-1 while highly susceptible to *Xoo*-2 and *Xoo*-3. The present study revealed that wild species of rice *O. longistaminata* and *O. rufipogon* were extremely resistant to all 3 local isolates of *Xoo* and therefore can be utilized for rice breeding advancement of rice germplasm available in Pakistan.

Discussion

In Pakistan bacterial blight has become a destructive disease of rice. Use of resistant cultivars is the most effective and efficient methods to control this plant disease. Whereas germplasm of rice available in the country is susceptible to virulent isolates of bacterial blight (Akhtar, 2005).

Three local isolates (*Xoo*-1, *Xoo*-2 and *Xoo*-3) collected, from different regions of Pakistan were used for the evaluation of cultivated varieties and wild species of rice.

High level resistance was shown by *Oryza rufipogon* among all the three isolates used in this study. This species hence proved to be the most valuable source of resistance to different *Xoo* isolates of Pakistan. *Oryza longistaminata* was also highly resistant to these isolates.

Cultivated varieties showed differential responses to these isolates. Bas-385 showed moderately susceptible reactions to *Xoo*-2 and *Xoo*-3. However it was moderately resistant to *Xoo*-1. JP-5 and F. Malakand were somewhat resistant to *Xoo*-1 and *Xoo*-2 while more or less susceptible to *Xoo*-3 only. Swat-1 was highly susceptible to all the three isolates. Results revealed that *Oryza rufipogon* can be utilized as resistance source to these isolates.

Table 4. Lesion size (as % of leaf length) of cultivated varieties and wild species of rice inoculated with different *Xoo* isolates

Varieties/Isolates	***Xoo*-1**	***Xoo*-2**	***Xoo*-3**
	Lesion size (%)	**Lesion size (%)**	**Lesion size (%)**
O. longistaminata	2	3	16
O. rufipogon	2	1	3
Bas-385	23	38	36
Swat-1	36	87	89
JP-5	18	13	29
FakhreMalakand	13	19	28

Table 5. Reactions of cultivated varieties and wild species of rice in response to different *Xoo* isolates

Varieties/Isolates	***Xoo*-1**	***Xoo*-2**	***Xoo*-3**
O. longistaminata	HR	HR	MR
O. rufipogon	HR	HR	HR
Bas-385	MR	MS	MS
Swat-1	MS	HS	HS
JP-5	MR	MR	MS
FakhreMalakand	MR	MR	MS

HR: Highly resistant, MR: Moderately resistant, HS: Highly susceptible, MS: Moderately susceptible

Resistance characterization of thirty randomly selected F3 (Bas-385 × *O. rufipogon*) genotypes showed that most genotypes were highly resistant to all the three isolates. These genotypes have successfully incorporated the resistance of donor *O. rufipogon* and can be further used in introgressive hybridization. Moreover, the resistance possessed by *O. rufipogon* is of dominant nature and of wide spectrum.

Bacterial blight disease can be controlled in safe mode through cultivation of resistant varieties (Waheed *et al.,* 2009). Although cultural practice is one the important tool to control this, but primary and most efficient control is planting of resistant cultivars.

Different races of same pathogen survive in the same field on the same cultivar (Vera Cruz *et al.*, 1996). Xoo population obtained from different districts of Indian Punjab has shown high degree of variety in pathogen population Different districts of Indian Punjab *Xoo* population obtained have been found high degree of variety in pathogen population. They observed that bacterial blight resistance gene *xa8* & *Xa21* are very useful against the established isolates in India followed by *xa5* and *Xa7* (Sodhi *et al.*, 2003). The Punjab province of Pakistan is closely adjacent to Indian Punjab province where the aromatic basmati rice grown, therefore it could possibly be infected by same gene pool of BB pathogen already seen in India. Research on pathogen populations in different countries and region explained that pathogen populations are diverse, which could be recognized by the slow progress/distribution of the pathogen or slow partitioning of host genotypes (Adhikari *et al.*, 1995; Leach *et al.*, 1992; Nelson *et al.*, 1994).

References

Adhikari TB, Vera CCM, Zhang Q, Nelson RJ, Skinner DZ, Mew TW, Leach JE. 1995. Genetic diversity of *Xanthomonas oryzae* pv. *oryzae* in Asia. Appl. Environ. Microbiol **61**, 966-971.

Akhtar MA. 2005. Studies on genetic variation in *Xanthomonas Oryzae* pv. *Oryzae* in relation to resistance in rice. In: 3rd annual progress report ALP-Project. Inst. Plg. and Env. Protect. National Agricultural Research Center, Islamabad, 31.

Akhtar MA, Zakria M, Abbassi FM, Masod MA. 2003. Incidence of bacterial blight of rice in Pakistan during 2002. Pakistan Journal Botany, **35(5),** 993-997.

Akhtar MA, Akram M.1987. Incidence of bacterial blight of rice in the Punjab, Pakistan, IRRN, **5**, 5.

Brar DS, Khush GS. 1997. Alien introgression in rice. Plant Mol. BioI. **(35)**, 35-47.

Chakravarthi BK, Naravaneni R. 2006. SSR marker based DNA finger-printing and diversity study in rice (*Oryza sativa* L.). African Journal of Biotechnology. **5(9)**, 684-688.

Chaudry RC. 1996. Standard evaluation system for rice. Genetic Res. Cent. Intl. Rice Res. Institute. Manila, Philippines, 52**.**

Eizenga GC, Agrama HA, Lee FN, Jia Y. 2009. Exploring genetic diversity and potential novel disease resistance genes in a collection of rice (*Oryza* spp) wild relatives. Gen. Resource. Genetic Resources and Crop Evolution. **56**, 65-76.

Ezuka A, Kaku H, 2000. A historical review of bacterial blight of rice. National Inst.
Agrobio. Res. Bull. Japan, 207.

IRRI (International Rice Research Institute). 1993. Rice Almanac. IRRI-WARDA-CIAT, Los Banos, Laguna, Philippines.

Khan TZ, Gill MA, Khan MG. 2000. Screening of rice varieties/lines for resistance to bacterial leaf blight, Pakistan Journal of Phytopathology. **12(1),**71-72.

Leach JE, Rhoads ML, VeraCruz CM, White FF, Mew TW, Leung H. 1992. Assessment of genetic diversity and population structure of *Xanthomonas oryzae* pv. *oryzae* with a repetitive DNA. Applied Environmental Microbiology. **58**, 2188–2195.

Mew TW. 1987. Current status of future prospects of research on bacterial blight of rice. Annual Review of Phytopathology, **25**: 359-382.

Mew TW, Majid A. 1977. Bacterial blight of rice in Pakistan. *IRRN*, **2**, 5-7.

Mew TW, Alvarez AM, Leach JE, Swings J. 1993. Focus on bacterial blight of rice. *Pl. Disease*, **77,** 5-12.

Nelson RJ, Baraoidan MR, VeraCruz CM, Yap IV, Leach JE, Mew TW, Leung H. 1994. Relationship between phylogeny and pathotype for the bacterial blight pathogen of rice. Applied Environmental Microbiology. **60,** 3275-3283.

Ou SH. 1985. *Rice diseases*. 2nd ed. Commonwealth Mycological Institute, Kew, Surrey, England, 61-96.

Sodhi M, Vikal Y, George MLC, Bala GS, Mangat GS, Garg M, Sidhu JS, Dhaliwal HS. 2003. DNA fingerprinting and virulence analysis of *Xanthomonas oryzae* pv. *oryzae* isolates from Punjab, northern India. Euphytica. **130 (1)** 107-115.

Swings J, Mooter MV, Vauterin L, Hoste B, Gills M, Mew TW, Kersters K. 1990.Reclassification of the causal agents of bacterial blight (*Xanthomonas campestris* pv. *oryzae*)and bacterial leaf streak (*Xanthomonas campestris* pv. *oeyzicol*) of rice as pathovars of *Xanthomonas oryzae* (ex. Ishiyama, 1922) sp. Now. Nom. Rev. International journal of systematic bacteriology. **40,** 309-311.

Tabie H, Mukoo H. 1960. Anatomical studies of rice plant leaves affected with bacterial leaf blight, in particular reference to the structure of water exudation system. Scholarly articles for Bull National Institute Agricultural Science. **11**, 37-43.

VeraCruz CM, Ardales DZ, Skinner JT, Nelson RJ, Louws FJ, Mew TW, Leach JE. 1996. Measurement of Haplotypic variation in *Xanthomonas oryzae* pv *oryzae* within a single field by rep PCR and RFLP analysis. Phytopathology. **86,** 1352-1359.

Waheed M, Inamullah A, Habib A, Sirajuddin K. 2009. *Xanthomonas Oryzae* pv. *Oryzae*. Pakistan. Journal of Bototany. **38(1)** 193-203.