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STUDIES ON VIRULENCE REACTIONS OF LOCAL ISOLATES OF *XANTHOMONAS ORYZAE* PV. *ORYZAE*

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Abstract

Local isolates of *Xanthomonas oryzae* pv. *oryzae* were collected from different rice producing areas of Pakistan and evaluated for their virulence on 12 rice lines viz., IRBB1, IRBB2, IRBB3, IRBB4, IRBB5, IRBB7, IRBB8, IRBB10, IRBB11, IRBB13, IRBB14 and IRBB21. The ability of an isolate to cause lesions with different lengths across the lines was interpreted as virulence. Isolates that were consistently associated with high or low virulence were differentiated. Isolates produced lesions of different sizes on different rice lines. Five virulence groups (races) were identified based on the virulence of the bacterial isolates on these lines. No single gene tested was found to be resistant against all virulence groups prevalent in Pakistan. The results of this study will facilitate the breeders in deployment of effective resistance genes against *X. oryzae* pv. *oryzae* strains prevalent in Pakistan.

Introduction

Bacterial leaf blight disease caused by *Xanthomonas oryzae* pv. *oryzae* is one of the most destructive diseases of rice throughout the world with the exception of Europe (Shanti *et al*., 2001). It is an economically important disease of rice in tropical Asia (Mew *et al*., 1979). The disease was reported from Pakistan in 1977 (Mew *et al*., 1989). Afterwards in 1987 its occurrence was confirmed from all the provinces of Pakistan (Akhtar *et al*., 2003). It has been observed during recent years that BLB disease incidence is increasing in Pakistan especially in "Kaller" belt, famous for rice cultivation (Khan *et al*., 2000). In the North West Frontier Province (NWFP), 20-25% disease incidence has been reported (Akhtar &Akram, 1987). Bacterial leaf blight has the potential to become a destructive bacterial disease of rice in Pakistan and can cause huge losses mainly because information regarding the pathogen and its effective control measures is lacking. Although no studies have been conducted to assess the losses caused by the disease in Pakistan, but it has been observed that disease appearance at early stages causes greater damage due to complete wilting (kresek) of the seedling. Generally BLB disease is more destructive in Asia during the heavy rains of monsoon season. The yield of rice may reduce up to 50% in fields that are severely infected. Usually plants are affected at the later stage of tillering, reducing rice yield from 10 to 20%. However infection of crop at tillering stage can sometimes lead up to 100% yield losses (Mew & Majid, 1977).

Strains of *X. oryzae* pv. *oryzae* have been classified into many races in different countries based on their virulence on near isogenic rice varieties. Differential systems have been presented from different countries that have significance in respective countries. However, in order to compare the pathogenic differentiation among different

countries, an international set of near isogenic rice lines was desirable. For that purpose an International Collaborative Research Project was initiated by the International Rice Research Institute (IRRI) Philippines in 1977. Three sets of differentials around single major resistance genes so far known with near-isogenic background of 3 cultivars viz., IR24, Toyonishiki and Milyang 23, were developed (Ezuka & Kaku, 2000).

In this study strains of *X. oryzae* pv. *oryzae* isolated from all rice growing areas of Pakistan were tested for their virulence on 12 near isogenic rice lines having Bacterial Leaf Blight resistance genes *Xa1+Xa12*, *Xa2*, *Xa3*, *Xa4*, *xa5*, *Xa7*, *xa8*, *Xa10*, *xa11*, *xa13*, *Xa14* and *Xa21* developed at IRRI, Philippines (Kinoshita, 1991). These lines were developed by backcrossing resistance sources to the recurrent parent IR24, which was susceptible to all known Philippine races of the pathogen (Ogawa *et al*., 1991). The main objective of this study was to identify the races of *X. oryzae* pv. *oryzae* prevalent in Pakistan based on their reactions on the near isogenic rice lines.

Materials and Methods

Sampling: Surveys for bacterial leaf blight disease were conducted in the 4 agro ecological rice zones of Pakistan between August and November during the years 2002 and 2003. Zone-1 includes the terraced valleys of NWFP such as lower Dir and Swat districts. Zone-2 comprises the areas of Punjab province such as Sargodha, Hafizabad, Sheikhupura, Sialkot, Narowal, Gujranwala, Gujrat, Lahore, Kasur and Okara districts. Zone-3 covers the land on the west bank of the river Indus including areas of Sindh province such as Larkana, Sheikarpur, Dadu, Nawab Shah, Jacobabad districts as well as few areas of Balochistan province including Nasirabad and Usta Muhammad districts. While zone 4 includes the areas of Thatta and Badin districts. In each district number of locations visited depended upon cropping intensity of rice. Zone-1 and zone-2 are densely cropped areas while zone-2 and 3 are scarcely cropped for rice. More locations and fields were visited in zone-1 and 2 while few fields were visited in zone-3 and 4. So number of foliar samples collected from zone-1 and 2 was greater than zone-3 and 4. For sampling, plants were observed at 5 points along a diagonal transect in a field. At each point four plants were examined for disease symptoms and upper 3 leaves of each plant were collected. These leaves formed composite and a representative leaf sample was taken for isolation of bacteria.

Isolation of bacteria: Bacteria were isolated from infected rice leaves by following the method of Adhikari *et al.*, (Adhikari *et al*., 1995). Bacteria that have yellow circular colonies with entire margins and smooth waxy and shiny surfaces were isolated. Single bacterial colonies of each strain were isolated. The isolated colonies were further purified on peptone sucrose agar (PSA) medium (Ou, 1985). Afterwards bacterial cells were preserved in 5% skimmed milk and PSA slants at 4^{o}C as a source for further work. For long-term storage, the isolates were preserved in silica gel at 4^{o}C. The bacterial strains were then inoculated onto the susceptible rice variety IR24 to verify their pathogenicity. The 105 bacterial strains isolated from field samples were confirmed to be *X. oryzae* pv. *oryzae* by pathogenicity test on the susceptible variety. Larger number of isolates was obtained from zone-1 (43 isolates) and zone-2 (52 isolates) because of more number of samples while few isolates were obtained from zone-3 (9 isolates) and zone-4 (One isolate) because of less number of samples. All the 105 bacterial isolates were named in a sequence from Xoo-1 to Xoo-105. Xoo stands for *X. oryzae* pv. *oryzae* (Table 1).

Table 1. Isolates of *Xanthomonas oryzae* pv. *oryzae* characterized for their reactions induced on near isogenic rice lines.

Isolate	District	Zone	Year	Variety	Isolate	District	Zone	Year	Variety
Xoo-1	Sargodha	2	2002	Super Basmati	Xoo-53	Lower Dir	1	2002	JP-5
Xoo-2	Sargodha	2	2002	Super Basmati	Xoo-54	Swat	1	2002	JP-5
Xoo-3	Hafizabad	2	2002	Super Basmati	Xoo-55	Swat	1	2002	JP-5
Xoo-4	Hafizabad	2	2002	Super Basmati	Xoo-56	Swat	1	2002	JP-5
Xoo-5	Hafizabad	2	2002	Basmati 385	Xoo-57	Swat	1	2002	JP-5
Xoo-6	Hafizabad	2	2003	Basmati 385	Xoo-58	Swat	1	2002	JP-5
Xoo-7	Hafizabad	2	2003	Basmati 385	Xoo-59	Swat	1	2002	JP-5
Xoo-8	Sheikhupura	2	2002	Super Basmati	Xoo-60	Swat	1	2002	JP-5
Xoo-9	Sheikhupura	2	2002	Super Basmati	Xoo-61	Swat	1	2002	JP-5
Xoo-10	Sheikhupura	2	2002	Super Basmati	Xoo-62	Swat	1	2002	JP-5
Xoo-11	Sheikhupura	2	2003	Super Basmati	Xoo-63	Swat	1	2002	JP-5
Xoo-12	Sheikhupura	2	2003	Super Basmati	Xoo-64	Swat	1	2002	JP-5
Xoo-13	Sheikhupura	2	2003	Super Basmati	Xoo-65	Swat	1	2002	JP-5
Xoo-14	Sheikhupura	2	2003	Super Basmati	Xoo-66	Swat	1	2002	JP-5
Xoo-15	Sheikhupura	2	2003	Super Basmati	Xoo-67	Swat	1	2002	JP-5
Xoo-16	Sialkot	2	2002	Basmati 386	Xoo-68	Swat	1	2002	JP-5
Xoo-17	Sialkot	2	2002	Basmati 386	Xoo-69	Swat	1	2002	JP-5
Xoo-18	Sialkot	2	2003	Basmati 386	Xoo-70	Swat	1	2002	JP-5
Xoo-19	Sialkot	2	2003	Basmati 386	Xoo-71	Swat	1	2002	JP-5
Xoo-20	Sialkot	2	2003	Basmati 386	Xoo-72	Swat	1	2002	JP-5
Xoo-21	Narowal	2	2002	Super Basmati	Xoo-73	Swat	1	2002	JP-5
Xoo-22	Narowal	2	2003	Super Basmati	Xoo-74	Swat	1	2002	JP-5
Xoo-23	Narowal	2	2003	Super Basmati	Xoo-75	Swat	1	2002	JP-5
Xoo-24	Gujranwala	2	2002	Super Basmati	Xoo-76	Swat	1	2003	JP5
Xoo-25	Gujranwala	2	2002	Super Basmati	Xoo-77	Swat	1	2003	JP5
Xoo-26	Gujranwala	2	2002	Super Basmati	Xoo-78	Swat	1	2003	JP5
Xoo-27	Gujranwala	2	2002	Basmati 386	Xoo-79	Swat	1	2003	JP5
Xoo-28	Gujranwala	2	2002	Basmati 385	Xoo-80	Swat	1	2003	JP5
Xoo-29	Gujranwala	2	2003	Basmati 385	Xoo-81	Swat	1	2003	JP5
Xoo-30	Gujranwala	2	2003	Basmati 385	Xoo-82	Swat	1	2003	JP5
Xoo-31	Gujranwala	2	2003	Basmati 385	Xoo-83	Swat	1	2003	JP5
Xoo-32	Gujrat	2	2002	Basmati 385	Xoo-84	Swat	1	2003	JP5
Xoo-33	Gujrat	2	2002	Basmati 386	Xoo-85	Swat	1	2003	JP5+Bas 385
Xoo-34	Gujrat	2	2003	Basmati 386	Xoo-86	Swat	1	2003	JP5+Bas 385
Xoo-35	Gujrat	2	2003	Super Basmati	Xoo-87	Swat	1	2003	JP5+Bas 385
Xoo-36	Gujrat	2	2003	Super Basmati	Xoo-88	Swat	1	2003	JP5+Bas 385
Xoo-37	Lahore	2	2002	Super Basmati	Xoo-89	Swat	1	2003	Bas 385
Xoo-38	Lahore	2	2002	Super Basmati	Xoo-90	Swat	1	2003	Shoga
Xoo-39	Lahore	2	2003	Super Basmati	Xoo-91	Swat	1	2003	Shoga
Xoo-40	Lahore	2	2003	Super Basmati	Xoo-92	Swat	1	2003	Shoga
Xoo-41	Lahore	2	2003	Super Basmati	Xoo-93	Swat	1	2003	Shoga
Xoo-42	Kasur	2	2002	Super Basmati	Xoo-94	Swat	1	2003	Shoga
Xoo-43	Kasur	2	2003	Super Basmati	Xoo-95	Malakand	1	2003	Shoga
Xoo-44	Kasur	2	2003	Basmati 385	Xoo-96	Larkana	3	2002	IRRI-6+ Shoga
Xoo-45	Kasur	2	2003	Basmati 385	Xoo-97	Larkana	3	2003	IRRI-6+ Shoga
Xoo-46	Kasur	2	2003	Basmati 385	Xoo-98	Larkana	3	2003	IRRI-6+ Shoga
Xoo-47	Kasur	2	2003	Basmati 385	Xoo-99	Larkana	3	2003	IRRI-6+ Shoga
Xoo-48	Kasur	2	2003	Basmati 385	Xoo-100	Larkana	3	2003	Rusi Basmati
Xoo-49	Kasur	2	2003	Basmati 385	Xoo-101	Larkana	3	2003	Rusi Basmati
Xoo-50	Okara	2	2002	Basmati 386	Xoo-102	Sheikarpur	3	2002	Rusi Basmati
Xoo-51	Okara	2	2002	Basmati 386	Xoo-103	Jacobabad	3	2002	IRRI-6
Xoo-52	Okara	2	2003	Basmati 386	Xoo-104	Jacobabad	3	2003	Sada Gulab
					Xoo-105	Badin	4	2003	Red Rice

Xoo = *Xanthomonas oryzae* pv. *oryzae*, Bas= Basmati

Table 2. Mean lesion lengths caused by five races of *Xanthomonas oryzae* pv. *oryzae* on near-isogenic rice lines in Pakistan

Rice line	Resistance gene	Races				
		35731 (PKX1)	73661 (PKX2)	75701 (PKX3)	75721 (PKX4)	77731 (PKX5)
IRBB1	*Xa1 + Xa12*	14.16 (S)	11.33 (S)	8.65 (S)	12.33 (S)	10.85 (S)
IRBB2	*Xa2*	13.89 (S)	12.01 (S)	8.54 (S)	12.21 (S)	10.71 (S)
IRBB3	*Xa3*	2.67 (R)	11.23 (S)	8.26 (S)	12.13 (S)	10.95 (S)
IRBB4	*Xa4*	14.25 (S)	11.75 (S)	8.43 (S)	12.31 (S)	10.76 (S)
IRBB5	*xa5*	2.55 (R)	11.52 (S)	1.87 (R)	2.11 (R)	10.65 (S)
IRBB7	*Xa7*	14.27 (S)	1.53 (R)	8.33 (S)	12.09 (S)	10.64 (S)
IRBB8	*xa8*	14.23 (S)	1.55 (R)	9.01 (S)	11.97 (S)	10.69 (S)
IRBB10	*Xa10*	14.51 (S)	11.57 (S)	8.77 (S)	11.75 (S)	10.86 (S)
IRBB11	*Xa11*	13.93 (S)	11.50 (S)	8.55 (S)	12.09 (S)	10.31 (S)
IRBB13	*xa13*	13.99 (S)	1.53 (R)	2.65 (R)	2.15 (R)	10.13 (S)
IRBB14	*Xa14*	14.27 (S)	11.70 (S)	2.58 (R)	12.51 (S)	10.11 (S)
IRBB21	*Xa21*	2.75 (R)	11.73 (S)	1.54 (R)	2.01 (R)	2.01 (R)
IR24	Xa16, Susceptible check	15.25 (S)	13.75 (S)	10.17 (S)	14.23 (S)	12.29 (S)
Total isolates		**12**	**53**	**20**	**8**	**12**

R= Resistant (<3cm length), and S= Susceptible (>3cm length)

Near-isogenic rice lines: Seeds of the near-isogenic rice lines and IR24 were procured from IRRI, Philippines. The experiments of *X. oryzae* pv. *oryzae* reactions were performed during the months of July-August 2004 at the National Agricultural Research Center, Islamabad, Pakistan. The 12 isogenic lines with genes, IRBB1 *(Xa1+Xa12)*, IRBB2 *(Xa2)*, IRBB3 *(Xa3)*, IRBB4 *(Xa4)*, IRBB5 *(xa5)*, IRBB7 *(Xa7)*, IRBB8 *(xa8)*, IRBB10 *(Xa10)*, IRBB11 *(xa11)*, IRBB13 *(xa13)*, IRBB14 *(Xa14)* and IRBB21 *(Xa21)* were used in this experiment (Table 2). IR24 was used as the susceptible check. Rice seeds were first sown in Petri plates in a growth chamber then after 10 days, 3 healthy seedlings of each line were transferred to 100 cm plastic buckets and were kept in glass house. The buckets were filled with mixture of soil and farmyard fertilizer (1:1). Moreover 10g mixture of nitrogen, phosphorus and potassium in the form of ammonium sulfate, superphosphate and muriate of potash was added to each bucket. The plants were watered every day and then 5g urea was added 30 days after transplantation to each bucket.

Inoculum preparation: The 105 strains of *X. oryzae* pv. *oryzae* isolated from the foliar samples of rice were incubated in PSA slants at 28^{o}C for 72 hours. The 3 day old cultures were then used to prepare inoculums. Inoculum was prepared by suspending separately the bacterial cells of each strain in 10ml of sterile distilled water and adjusting the concentration upto 10^{8} Colony Forming Units (CFU)/ml.

Inoculation: To test the virulence of bacterial strains on the near- isogenic rice lines, the experiment was designed in a split plot manner where the isogenic lines were considered as the main plot and bacterial isolates as subplots (Gomez & Gomez, 1984). Thus the experimental unit consisted of 3 plants per strain inoculation (Adhikari *et al.*, 1999). Three plants of each isogenic line were inoculated with each of the 105 strains of *X. oryzae* pv. *oryzae* using clip inoculation method 40 days after sowing (Kauffman *et al*., 1973). For this purpose 1 to 2 cm of the tips of three fully expanded leaves of each plant in each bucket (total of nine leaves per strain inoculation) were clipped with scissors dipped in inoculum. Control plants were inoculated similarly with sterilized distilled water.

Estimation of infection: After 14 days of inoculation the lengths of lesions were measured in centimeters from the cuts of leaf tips (Mew *et al*., 1989). Resistance or susceptibility was assessed from the mean lesion length of the 9 inoculated leaves of each isogenic line. Reactions of lines were categorized according to lesion length, where 0 to 3 cm length was classified as resistant (R) and more than 3 cm as susceptible (S) (Adhikari *et al*., 1999).

Race analysis: Lesion length data for each line – isolate combinations were subjected to HaGis race analysis software package (Version 3.1). HaGis is a tool (developed in Microsoft Excel 97, which combines the user-friendly interface of Windows) for input and analysis of experimental data. For input of results based on lesion lengths, susceptibility was coded as "1" and resistance as "0". The programme is based upon Habgood & Gilmour race analysis and race naming methods (Gilmour, 1973; Habgood, 1970). Habgood &Gilmour names are calculated following Limpert & Muller (1994) by keeping the order of isogenic lines as listed in the data set Table and weighing with increased powers of 2, 3 and 4, respectively. Gilmour names were represented in the form of an octal code (Herrmann *et al.*, 1999). For naming of *X. oryzae* pv. *oryzae* races Gilmour names were adopted in this study. The distribution of these races among the 105 isolates was calculated by the formula of Herrmann *et al*., (1999). For each race of *X. oryzae* pv. *oryzae* virulence difference was also calculated. Virulence difference is defined as the number of isogenic lines that exhibit a different disease reaction compared to the most frequent (dominant) race (Roelfs and Groth, 1980). The susceptibility of each isogenic line was also calculated from the observed number of isolates, which were susceptible to the line (Zhang, 1987). Pathogenicity index of each gene was calculated by adding up the assessment ratings *ai* of all isolates in the sample and dividing them by the product max. N of the maximum assessment value max and the number N of isolates, i.e. PI= ∑ai/ (max. N) (Zhang, 1987). The proximity matrix for pathotype pairs was calculated based on a 2 x 2 contingency Table using following simple match formula (Johnson & Wichern, 1992).

$$\text{Simple match (\%)} = 100.\ (a + d) / (a + b + c + d)$$

Results

Five races of local isolates of *Xanthomonas oryzae* pv. *oryzae* were found using HaGis analysis software. The codes of these races were 35731, 73661, 75701, 75721 and 77731 according to Gilmour octal representation. For convenience these races were further named as PKX1, PKX2, PKX3, PKX4 and PKX5 respectively because it is difficult to remember the codes. PK stands for Pakistan and X stands for *X. oryzae* pv. *oryzae* (Table 2). The isolates belonging to race PKX1 were virulent to resistance genes *Xa1+Xa12, Xa2, Xa4, Xa7, xa8, Xa10, Xa11, Xa13, Xa14,* and *Xa16* but were avirulent to genes *Xa3, xa5* and *Xa21.* Race PKX2 was found virulent to resistance genes *Xa1+Xa12, Xa2, Xa3, Xa4, xa5*, *Xa10, Xa11, Xa14, Xa21* and avirulent to genes X*a7, xa8* and *xa13.* Race PKX3 exhibited virulence to genes *Xa1+Xa12, Xa2, Xa3, Xa4, Xa7, Xa8, Xa10, Xa11* and avirulence to genes *xa5, xa13, Xa14* and *Xa21.* Race PKX4 was virulent to genes *Xa1+Xa12, Xa2, Xa3, Xa4, Xa7, xa8, Xa10, Xa11* and *Xa14* and avirulent to genes *xa5, Xa13* and *Xa21.* Race PKX5 was virulent to all genes except *Xa21.* The distribution

of these races among the 105 isolates was calculated and it was found that out of the total 105 isolates of *X. oryzae* pv. *oryzae* race PKX1 was distributed among 12 isolates, whereas PKX2, PKX3, PKX4 and PKX5 were found among 53, 20, 8 and 12 isolates respectively. From the distribution of races, frequency of each race was estimated. The race which was distributed among maximum number of isolates was considered to be most frequent and a dominant race. By comparing the frequencies of the 5 races among the isolates of *X. oryzae* pv. *oryzae*, it was observed that race PKX2 was most frequent and hence was the dominant race while PKX4 was the least frequent race (Fig. 1).

Gene *Xa21* was resistant to all races except race 73661 (PKX2) that was the most frequently distributed race (Table 2). Moreover it was observed that only a single isogenic line having gene *Xa21* was resistant to race 77731 (PKX5) while all other lines were susceptible to it.

While calculating virulence difference of the races it was found that PKX2 had 0 virulence difference value since it was the most frequent race whereas the virulence differences of races PKX5, PKX4, PKX3 and PKX1 were 4, 4, 5 and 6 respectively. The distribution of virulence differences of the five local races of *Xanthomonas oryzae* pv. *oryzae* are given in (Fig. 2).

The more the number of isolates that gave susceptible reactions on an isogenic line more was the susceptibility of that line. The genes *Xa1, Xa12, Xa2, Xa4, Xa10, Xa11,* and *Xa16* present in the lines IRBB1, IRBB2, IRBB4, IRBB10, IRBB11 and IRBB21 respectively were 100% susceptible while genes *Xa3, xa5, Xa7, xa8, xa13, Xa14* and *Xa21* present in the lines IRBB3, IRBB5, IRBB7, IRBB8, IRBB13, IRBB14 and IRBB21 were found to be 89%, 62%, 50%, 50%, 23%, 81% and 50% susceptible (Fig. 3).

The percentage pathogenicity index of genes *Xa1* and *Xa12* combination was 100%, whereas those of *Xa2, Xa3, Xa4, xa5, Xa7, xa8, Xa10, Xa11, xa13, Xa14, Xa21* and *Xa16* were 100%, 88.6%, 100%, 61.9%, 49.5%, 49.5%, 100%, 100%, 22.9%, 81.0%, 50.5% and 100% respectively.

The proximity matrix for pathotype pairs was calculated. Similarities of race PKX1 to the races PKX2, PKX3, PKX4 and PKX5 were 70%, 84%, 90% and 91% respectively whereas the similarities of race PKX2 to the races PKX3, PKX4 and PKX5 were 74%, 80% and 82% respectively. However similarities of race PKX3 to races PKX4 and PKX5 were 95% and 86% respectivelyw while the races PKX4 and PKX5 were 91% similar (Table 3) (Fig. 3).

The distribution of different races among all the four agro-ecological rice zones, was also compared. It was found that race PKX2 was widely distributed in zone-2 comprising 38 isolates (73%) out of the total isolates obtained from this zone, whereas it included 14 isolates (32%) from zone-1 but in this race only 1 isolate (12%) was from zone-3. Race PKX2 was absent from zone-4. Race PKX5 comprised 8 isolates (15%) from zone-2, 2 isolates (5%) from zone-1 and 2 isolates (22%) from zone-3. Race PKX4 consisted of 6 isolates (12%) from zone-2, 2 isolates (5%) from zone-1 and no isolate (0%) from zone-3 and 4. Race PKX3 was not found in zone-2 and 4, while it included 17 isolates (40%) from zone-1 and 3 (33%) out of zone-3 isolates. Race PKX1 was absent from zone 2 while it had 8 isolates (18%) out of zone-1 and 2 isolates whereas 3 isolates (33%) from zone-3. From zone-4 the only isolate obtained belonged to race PKX1 (Fig. 4).

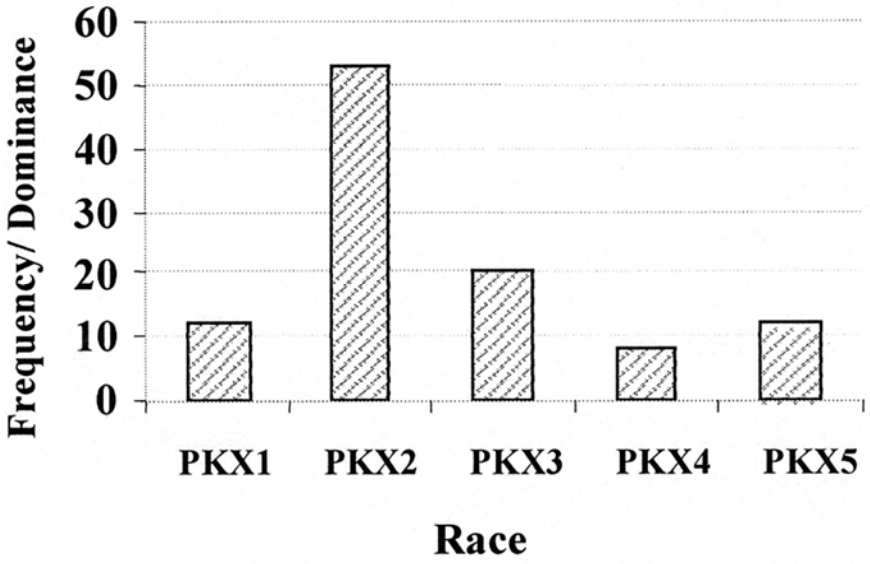

Fig. 1. Frequencies/ relative dominance of Pakistani races of *X. oryzae* pv. *oryzae* based on their distribution among 105 isolates

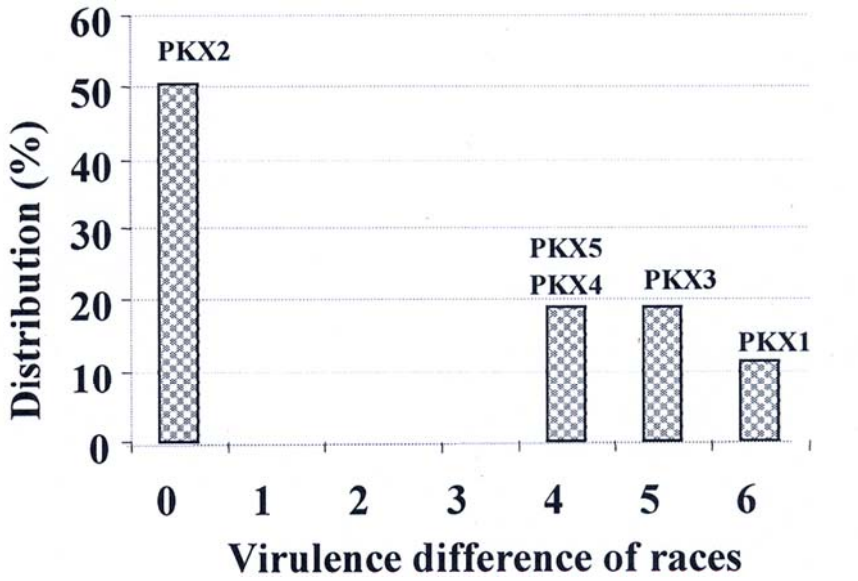

Fig. 2. Agro ecological rice zones of Pakistan and percentage distribution of *X. oryzae* pv. *oryzae* isolates from different zones among the five races.

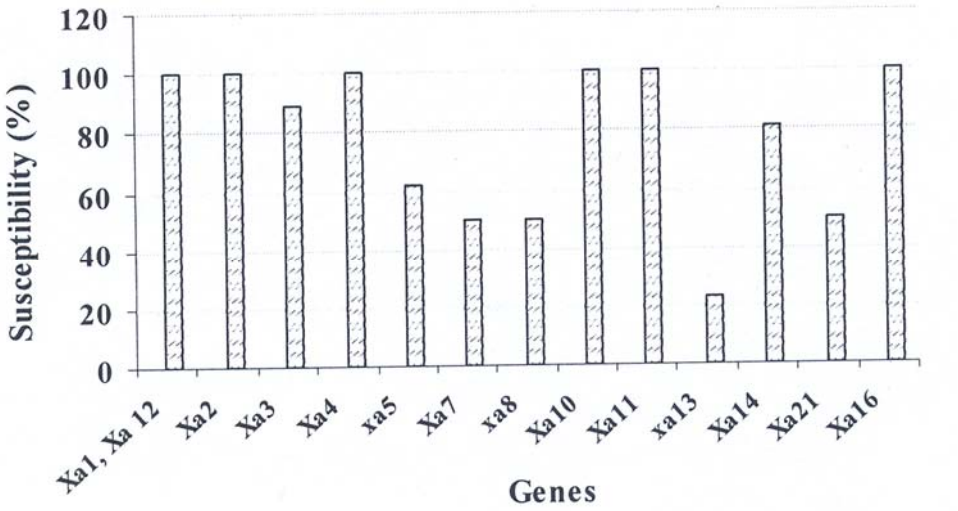

Fig. 3. Susceptibilities (%) of genes of near isogenic rice lines used to test virulence of Pakistani isolates of *X. oryzae* pv. *Oryzae*.

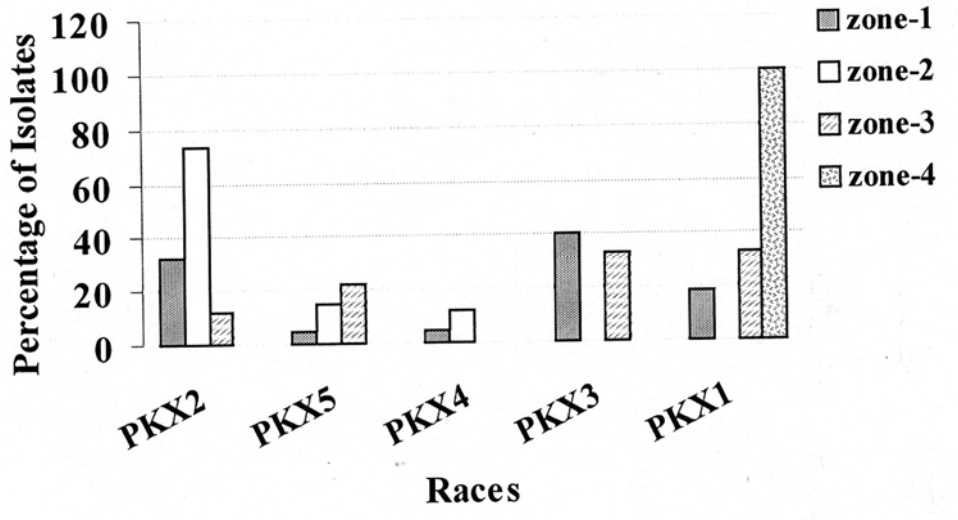

Fig. 4. Percentage distribution of Pakistani races of *X. oryzae* pv. *Oryzae* in different zones.

Discussion

Near-isogenic lines having different major genes for resistance to *X. oryzae* pv. *oryzae* were used to analyze virulence of 105 isolates of *X. oryzae* pv. *oryzae* in Pakistan. The lesions induced by the isolates were clear and easily classified into virulent or avirulent on the test rice lines possessing different genes for resistance. The HaGis programme was used for analysis of virulence data of *X. oryzae* pv. *oryzae* strains because it is reliable and easy to use. The programme is helpful in assigning races of

pathogens whose populations consist of variants detected by their differential virulence on selected sets of host cultivars (Johnson, 1999). Moreover it is convenient for data entry and offers a first overview of the data by means of graphical and numerical representation. It displays the input data and offers a choice of distinct figures, tables, indices and statistics (Herrmann *et al*., 1999).

From this study it was found that none of the lines used was resistant against all the strains of *X. oryzae* pv. *oryzae* prevalent in Pakistan. Although *Xa21* is reported to be effective and stable against multiple isolates of *X. oryzae* pv. *oryzae* (Shanti *et al*., 2001), in this study the isolates belonging to race PKX2 were virulent to *Xa21*, which is the dominant race in Pakistan while the other 4 races PKX1, PKX3, PKX4 and PKX5 were avirulent to this gene. *X. oryzae* pv. *oryzae*, strains virulent to *Xa21* had also been reported from Japan (Lin *et al*., 1996), Korea (Lee *et al*., 1999), Nepal (Adhikari *et al*., 1999), Sri Lanka (Ochiai *et al*., 2000) and India (Goel *et al*.) Avirulence of most South Asian strains and many Korean strains of *X. oryzae* pv. *oryzae* on *Xa21* implies that *avrXa21* is widely distributed in Asian populations of *X. oryzae* pv. *oryzae*. However, the virulence of *X. oryzae* pv. *oryzae*, strains to *Xa21* might be the result of a mutation in *avrXa21* gene (Lee *et al*., 1999). The *Xa4* gene had also been widely used for resistance in tropical Asia. It was however found to be susceptible to all the 5 races distributed in all the rice zones in Pakistan. Same results were found in Nepal, where all the virulence-groups were found to be virulent to *Xa4* gene (Adhikari *et al*., 1999). *Xa4* conveyed resistance to the prevalent races in East and South Asia but not to those in South Asia. Therefore pathotypes overcoming *Xa4* gene in the Philippines (Ardales *et al*., 1996) India (Pandey *et al*., 1986) and Sri Lanka (Ochiai *et al*., 2000) have also been reported. The resistance gene *xa5* was resistant against 3 races PKX1, PKX3 and PKX4 while the gene *xa13* was resistance against 3 races PKX2, PKX3 and PKX4. Strong and broad resistance of *xa5* has been reported from other Asian countries such as Korea (Jeung *et al*., 2006), India (Shanti *et al*., 2001) and Sri Lanka (Ochiai *et al*., 2000). From these studies it is concluded that none of the single resistant genes used can control BLB disease in Pakistan. However a pyramid line containing genes *xa5*, *Xa13* and *Xa21* would be the most promising and valuable genotype for improving Pakistani cultivars for bacterial blight resistance. These findings are useful to rice breeding programs designed to develop stable broad-spectrum resistance to bacterial blight in rice cultivars.

Comparing the races of *X. oryzae* pv. *oryzae* in Pakistan with that of other Asian countries, it was found that race PKX5 was similar to the race 3 of Sri Lanka (Ochiai *et al*., 2000), since these races were virulent to all genes except *Xa21*. Overall Pakistani races of *X. oryzae* pv. *oryzae* resemble the races of other South Asian countries regarding their reactions to the major resistant genes but still they are distinct in their responses.

Considering the distribution of the 5 races among the rice zones of Pakistan, the origin of races PKX1 and PKX3 reveal association with shoga variety (Mutant of IR8, *Xa11*) and JP-5 variety (Japonica type) cultivated in zone-1 and zone-3. This race was absent in zone-2 due to change in rice varieties. Race PKX1 however was transmitted to zone-4 through contaminated floodwater. Race PKX2 was the most dominant race whose origin shows linkage to the basmati varieties (Indica type, genetic background of IR24) as it is highly prevalent among the isolates of zone-2 where basmati varieties are the major varieties grown, moreover in zone-1 and 3 it is found in those locations where the basmati varieties are cultivated with other varieties. PKX4 was found in zone-1 and 2. PKX5 was found in zone-1, 2 and 3. However its percentage prevalence was high in

zone-3 where mixed varieties (IRRI-6, Rosi Basmati, Red rice and sada gulab) are cultivated. These results indicate variability in virulence among the bacterial isolates and great complexity in the susceptibility of rice cultivars to the bacterial isolates. This shows that although the local populations of the pathogen are dynamic and diverse in Pakistan but are regionally dominated by specific races. A similar trend of geographic differentiation as well as population shifts of the rice bacterial blight pathogen population has been observed in Nepal (Adhikari *et al*., 1994), Philippines (Ardales *et al*., 1996) and Korea (Dardick *et al*., 2003).

By calculating the frequency of races it was found that race PKX2 is most frequent and hence dominant race in Pakistan. This shows that race PKX2 has more propensity to prevail in Pakistan. Moreover by the virulence difference the races PKX5 and PKX4 are closer to the most dominant race in their reactions. This indicates that these races have the tendency to become dominant.

With the help of susceptibility calculations we were able to find the genes that are susceptible to maximum number of Pakistani isolates of *X. oryzae* pv. *oryzae*. The genes Xa1, Xa12, xa2, Xa4, Xa10, xa11 and Xa14 are susceptible to all the isolates. These genes are not effective for resistance purposes instead the genes that show minimum susceptibility including the genes Xa7, Xa8 and Xa13 must be used in breeding of resistant varieties.

From proximity matrix it was found that race PKX3 and PKX4 were the most similar races having 92% similarity. Moreover these 2 races show the same virulence difference value that is 4. From this it is inferred that race PKX4 was recently originated by mutation in PKX3 at zone 1, since only 8 isolates belonged to race PKX4. In this evolutionary process the new strains became more virulent and break the resistance of *Xa14* gene that was resistant to PKX3. This shows that new pathotypes of *X. oryzae* pv. *oryzae* continue to evolve that can overcome the resistance conveyed by the major genes.

The results of this analysis are also useful in selection of *X. oryzae* pv. *oryzae* strains for further resistance screening. Hence, it is suggested that race PKX2 and PKX5 would be the best choice to evaluate resistance in Pakistani rice varieties. Because PKX2 is the only race that is virulent to *Xa21* gene and PKX5 is the race virulent to all the genes used except *Xa21*. However, because of the pathogen's ability to rapidly overcome major genes, one of the most challenging researches will be to develop a sound strategy for deployment of R-genes in order to maximize the durability of resistance. Moreover regular monitoring of *X. oryzae* pv. *oryzae* population to assay the occurrence of the pathogenic races is also required for management of bacterial leaf blight disease in Pakistan.

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