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Full Length Research Paper

Identification of bacterial blight resistance genes *Xa4* in Pakistani rice germplasm using PCR

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Bacterial blight (BB) caused by *Xanthomonas oryzae* pv *oryzae (Xoo)* is a major biotic constraint in the irrigated rice belts. Genetic resistance is the most effective and economical control for bacterial blight. Molecular survey was conducted to identify the rice germplasm/lines for the presence of *Xa4*, a bacterial blight resistance gene. PCR with primers specific for *Xa4* resistances gene was used in the study. During this polymorphic survey, out of 100 rice germplasm lines obtained from NARC (IABGR), 49 lines were observed with *Xa4* gene. While 51 germplasm showed the absence of *Xa4* gene. Of the nineteen basmati breeding lines, obtained from Rice Research Station Kala Shah Kaku (KSK RRI), 7 lines (KSK1, KSK4, KSK6, KSK7, KSK8, KSK12 and KSK16) showed the presence of *Xa4* gene. The Pakistani released Basmati varieties were also surveyed. Of the eight Pakistani basmati varieties used, Basmati 198, Basmati 385, Basmati 2000 and Shaheen Basmati have the *Xa4* gene. The identification of *Xa4* gene in Pakistani rice germplasm will help in accelerating the elite breeding program in future, including pyramiding of different disease resistant genes in basmati varieties.

Key words: Rice, germplasm, Xa4, bacterial blight.

INTRODUCTION

The bacterial blight disease caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) has been one of the major factors limiting rice production in Asia (Mew, 1987). Yield loss of 26% on susceptible rice cultivars from the disease has been reported. The exploitation of host resistance has been shown to be the only reliable method to control the disease. So far, 27 genes exhibiting resistance against various strains of *Xoo* prevalent in Philippines, China, India, Japan, and Korea have been identified and named from *Xa*-1 to *Xa*-27 (Kinoshita, 1995; Zhang et al., 1998; Lin et al., 1996; Chen et al., 2002; Gu, et al., 2004). Fourteen of the 27 resistant genes which include *Xa-1* to *xa-5, Xa-7, xa-8*, *Xa-10*, *Xa-12* to *Xa-14, Xa-21* to *xa-23*, *Xa-25*(t) and *Xa-27* have been mapped to chromosomes 4, 5, 6, 7, 8, 11 and 12 (Zhang et al., 1998; Lin et al., 1996; Kinoshita, 1995, Singh et al., 2002; Chen et al., 2002; Gu, et al., 2004). The identification and the characterization of major genes for qualitative resistance and polygenic factors controlling quantitative resistance have contributed a great deal to the success in breeding resistant cultivars. Many of these identified genes have been incorporated into modern rice varieties and exhibited complete resistance against the pathogens (Khush et al., 1989, Huang et al., 1997; Sanchez et al., 2000).

Bacterial blight resistance gene *Xa4* is one of the most widely exploited resistance gene in many Asian rice breeding programs and conferred durable resistance in many commercial rice cultivars (Mew et al., 1992). *Xa4* gene was identified by Petpisit et al. (1977), and rice cultivars carrying the gene are resistance to bacterial blight at all stages of plant growth and had been widely used in the breeding program (Khush 1981). Cultivars with *Xa4* are resistant to most types of *Xoo*. The pyramided lines with *Xa4* and other bacterial blight resistance genes showed a wider spectrum and a higher level of resistance than the lines with single resistance gene (Huang et al., 1997; Zheng et al., 1998). The gene was

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transferred into rice variety IR24 through repeated backcrossing resulting in the production of a near-isogenic line IRBB4 carrying *Xa-4* in IR24 genetic background (Ogawa et al., 1991).

Several molecular studies were conducted to identify the tightly linked markers for PCR based detection of *Xa4* gene in rice germplasm. Yoshimura et al. (1992) first mapped the *Xa4* gene on chromosome 11 linked to the RFLP marker G181. Later on Li et al. (1999) mapped it between markers RZ536 and G2132b. The objective of our study was to survey the rice germplasm and basmati Varieties for the presence of *Xa4* gene using specific primers.

MATERIALS AND METHOD

Plant materials

Seeds of the 100 rice genotypes/lines (list given in the (Table 1) along with their accession # and local names) obtained from Institute of Agricultural Biotechnology and Genetic Resources (IABGR), National Agriculture Research Council (NARC), Islamabad, with 19 basmati breeding lines (collected from Rice Research Institute Kala Shah Kaku), 8 commercial Basmati varieties viz., Basmati 370, Super Basmati, Basmati 385, Basmati Pak., Basmati 2000, Basmati 198, Kashmir Basmati and Shaheen Basmati and 3 IRRI varieties IRBB4, IR64 (having *Xa4* gene) and IR24 (with no *Xa4* gene) were grown in pots at National Institute for Biotechnology and Genetic Engineering (NIBGE), Faisalabad.

PCR amplification of *Xa4*

Young leaves at seedling stage were harvested for the isolation of genomic DNA. Fresh leaves from 5 individuals of each line/variety were bulked together and the DNA was extracted by following the protocol of Dellaporta et al. (1983). The concentration of extracted genomic DNA was measured by flourometer DyNA Quant^{TM}200 and the DNAs were diluted to 10 ng/uL using sterilized distilled water and stored in microfuge tubes at 4°C for further use.

Amplification of *Xa4* linked DNA fragment was carried out using specific primers (developed by Ma et al., 1999). Amplification reactions were carried out in 25 uL reaction volumes containing 50 ng genomic DNA, 1.0 µM each of primer MP1 (5'-ATCGATCGATCTTCACGAGG-3') and MP2 (5'-dTGCTATAAAAG-GCATTCGGG-3'), 100 uM each of dATP, dCTP, dGTP and dTTP, 1 unit of Taq DNA Polymerase (Fermentas), 1X Taq Polymerase Buffer and 2.5 mM $MgCl_2$. DNA amplification was performed in DNA Thermal Cycler (Eppendorf) programmed as follows: an initial denaturation of 5 min at 94°C; 35 cycles of 94°C for 1 min (denaturation), 55°C for 1 min (annealing), and 72°C for 2 min (extension). One additional cycle of 10 min at 72°C was used for final extension. Amplification products were resolved by electrophoresis on 1.5% agarose gels run in 0.5X TAE. The amplified products were observed under UV transilluminator after stained with ethidium bromide (10 ug/mL) and scored for the presence and absence of *Xa-4* linked DNA fragment.

Data analysis

The amplified fragment of all the rice genotypes/lines, basmati breeding lines and the basmati varieties were observed and compared with IRBB4 and IR24 for the presence (+) and absence (-) of *Xa4* gene.

RESULTS AND DISCUSSION

DNA analysis of all the rice germplasm, basmati breeding lines and different basmati varieties exhibited two different sizes of band. The banding pattern of all the individuals were either identical with that of the IRBB4 and IR64 (having *Xa4* gene) or with that of the IR24 (with no *Xa4*) gene. The size of the band corresponds to IR64 and IRBB4 is 150 bp whereas the band corresponds to IR24 is 120 bp in size. Ma et al. (1999) identified and synthesized this set of PCR primers based on the sequence of a DNA marker tightly linked to the rice bacterial blight (BB) resistance gene *Xa*4 for the survey of hybrid rice germplasm. Wang et al. (2000) used the same set of primer for the fine mapping of *Xa4* gene. They analyzed F2 population of cross between IR24 and IRBB4 using the same primers and found that *Xa4* is tightly linked to this marker.

During this polymorphic survey, out of 100 rice lines, 49 rice lines along with IRBB4 and IR64 amplified 150 bp size fragments indicating the presence of *Xa4* gene (Table 1). While the remaining 51 rice lines found to be without *Xa4* gene as 120 bp DNA fragment was found to be amplified in all these lines and also in IR24 (Figure 1). Similar type of molecular survey has been conducted by Ramalingam et al. (2001) for the presence of bacterial blight resistance genes *xa-5, xa-13* and *Xa-21* in Chinese rice germplasm. Although conventional approach for the identification of different resistance genes in rice germplasm is also being used (Lee et al., 2003; Kihupi et al., 2001), but it is time consuming and need artificial inoculation of all the lines with different pathotypes of the pathogen.

Of the nineteen-basmati breeding lines, 7 lines i.e. KSK1, KSK4, KSK6, KSK7, KSK8, KSK12 and KSK16 showed the presence of *Xa4* gene (Figure 1). This implies that these basmati breeding lines are the source of *Xa4* gene, which could be transferred to different basmati varieties during the crossing and selection procedure. In this study, of the eight Pakistani Basmati varieties used, Basmati 198, Basmati 385, Basmati 2000 and Shaheen Basmati possess the 150 bp band corresponding to *Xa4* gene. Moreover, it is also noted that in most of the rice growing areas where the incidence is very high, Basmati 385 and Basmati 2000 showed resistance and developed less disease as compared to other Basmati varieties (Personal Communication). Khan et al. 2000 screened some Pakistani basmati varieties alongwith some mutant Basmati lines against the virulent strain *X. oryzae* prevailed in Pakistan, and found only Basmati 370 showing some resistance against *X. oryzae*. The observation indicates that the experimental strains and the strain causing disease in the field are different from each other and also different strains are prevailing in different rice growing areas. Vera Cruz et al. (1996) observed in their study that different races of the same pathogen exist in the same field on the same cultivar. It is hypothesized that the susceptibility of

Table 1. Rice genotypes/lines used in genetic analysis studies showing presence (+) and absence (-) of *Xa*4 gene.

S/N	Varieties/lines code	Acc. no.	Local name	*Xa4*	S/N	Varieties/lines code	Acc. no.	Local name	*Xa4*
1	MB-1	Pak 0244	Jhona 426-37	+	51	MB-51	Pak 0424	Palman 188	+
2	MB-2	Pak 0253	Santhi sufaid	+	52	MB-52	Pak 0425	Sufaida 246	-
3	MB-3	Pak 0255	Jhoni 213	+	53	MB-53	Pak 0428	Basmati 502	-
4	MB-4	Pak 0257	Dhan 263	+	54	MB-54	Pak 0429	Mutant 11-9	+
5	MB-5	Pak 0260	Dhan 400	+	55	MB-55	Pak 0432	Nc1 –536	+
6	MB-6	Pak 0262	TIRI 424-2	-	56	MB-56	Pak 0438	Ratua 3882	-
7	MB-7	Pak 0263	TIRI 429-3	+	57	MB-57	Pak 0440	Ratua 69	-
8	MB-8	Pak 0264	1A	+	58	MB-58	Pak 0445	Dhan Munji 238	-
9	MB-9	Pak 0265	3	+	59	MB-59	Pak 0448	Bamla suffaid	-
10	MB-10	Pak 0268	6	-	60	MB-60	Pak 0450	Sathra 338 A4	-
11	MB-11	Pak 0272	11	+	61	MB-61	Pak 0452	Sathra surkh	-
12	MB-12	Pak 0279	18A	+	62	MB-62	Pak 0457	Son 15	+
13	MB-13	Pak 0282	20	+	63	MB-63	Pak 0462	91 S2	-
14	MB-14	Pak 0287	24	-	64	MB-64	Pak 0463	16S-JHONA	+
15	MB-15	Pak 0289	24A-10	+	65	MB-65	Pak 0467	Munji sufaid	-
16	MB-16	Pak 0292	29A-1	+	66	MB-66	Pak 0468	170	-
17	MB-17	Pak 0297	31	+	67	MB-67	Pak 0469	Jhona Desi 185	-
18	MB-18	Pak 0298	32	-	68	MB-68	Pak 0472	Dhan 300	-
19	MB-19	Pak 0305	38	-	69	MB-69	Pak 0474	Sathra 343	-
20	MB-20	Pak 0308	40	+	70	MB-70	Pak 0475	345	-
21	MB-21	Pak 0309	41	-	71	MB-71	Pak 0476	368	-
22	MB-22	Pak 0312	43	-	72	MB-72	Pak 0479	Santhi sufaid	-
23	MB-23	Pak 0315	45	+	73	MB-73	Pak 0481	Santhi 232	-
24	MB-24	Pak 0317	52	+	74	MB-74	Pak 0482	Sathi Kalri 235	-
25	MB-25	Pak 0318	70	+	75	MB-75	Pak 0483	Santhi sufaid	-
26	MB-26	Pak 0319	71	+	76	MB-76	Pak 0484	Santhi 243	+
27	MB-27	Pak 0322	73	+	77	MB-77	Pak 0485	Santhi 256	-
28	MB-28	Pak 0324	75	+	78	MB-78	Pak 0487	Santhi 288	+
29	MB-29	Pak 0325	76	+	79	MB-79	Pak 0488	Santhi 290	-
30	MB-30	Pak 0328	80	+	80	MB-80	Pak 0489	Santhi 290A	+
31	MB-31	Pak 0331	81B	+	81	MB-81	Pak 0490	Sathra 252A	-
32	MB-32	Pak 0342	93	+	82	MB-82	Pak 0498	Sathra 305	-
33	MB-33	Pak 0344	SM3-34	+	83	MB-83	Pak 1763	-	-
34	MB-34	Pak 0347	SM6-34	+	84	MB-84	Pak 1764	Cheeni	-
35	MB-35	Pak 0349	SM9-36	+	85	MB-85	Pak 1768	Khanduri	-
36	MB-36	Pak 0350	SM12-34	+	86	MB-86	Pak 1772	Cheeni	+
37	MB-37	Pak 0351	SM16-34	+	87	MB-87	Pak 1775	Cheeni	-
38	MB-38	Pak 0355	Kharsu 80A	-	88	MB-88	Pak 1777	Chingan	-
39	MB-39	Pak 0363	Dhan 247	-	89	MB-89	Pak 2717	-	-
40	MB-40	Pak 0365	Dhan 263	+	90	MB-90	Pak 2783	-	+
41	MB-41	Pak 0366	Kharsu 295A	+	91	MB-91	Pak 2787	-	-
42	MB-42	Pak 0374	Mushkan 36-1	-	92	MB-92	Pak 2830	Brinj	+
43	MB-43	Pak 0379	Mushkan 56	-	93	MB-93	Pak 2836	Brinj	-
44	MB-44	Pak 0380	Mushkan 73S	+	94	MB-94	Pak 2862	Murgi brinj	+
45	MB-45	Pak 0382	Mushkan 77	+	95	MB-95	Pak 2873	Murgi brinj	-
46	MB-46	Pak 0383	Mushkan chahi	-	96	MB-96	Pak 2874	Brinj	-
47	MB-47	Pak 0394	Chambu 128	-	97	MB-97	Pak 2925	Chawal	+
48	MB-48	Pak 0395	Chahora 144	-	98	MB-98	Pak 2967	Kharay ganjay	-
49	MB-49	Pak 0398	Patti 168	+	99	MB-99	Pak 3375	Nali	+
50	MB-50	Pak 0409	Bara	-	100	MB-100	Pak 3402	Chinese	-

Accession # and local names are the same as mentioned in Plant Germplasm catalogue 1997, published by Institute of Agro.Biotechnology and Genetic Resources [previously Plant Genetic Resources Institute (PGRI)], NARC, Islamabad.

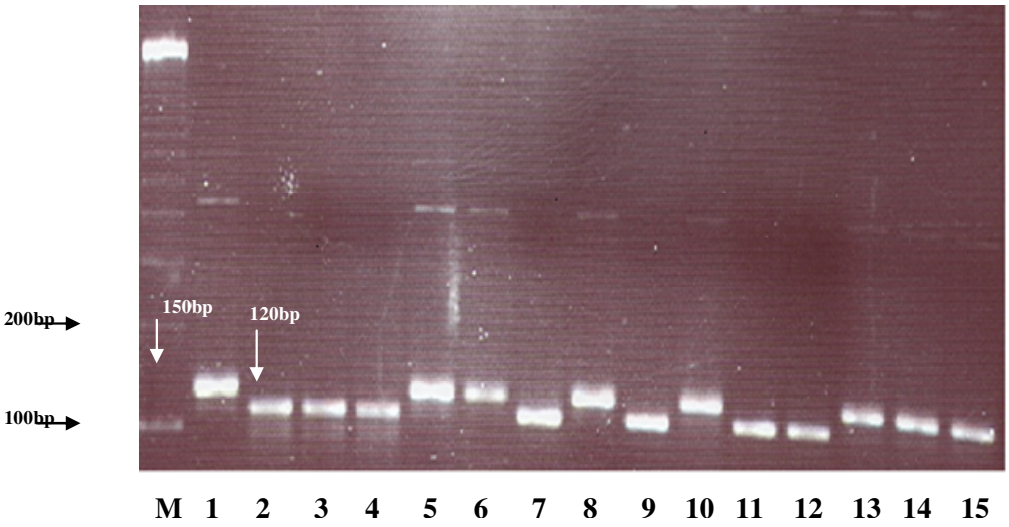

Figure 1a. Banding patterns showing the presence and absence of *Xa-4* gene in germplasm of rice amplified 150 bp and 120 bp size fragments respectively. Lane M = 100bp DNA ladder, Lane 1= IRBB-4, Lane 2 = IR-24, Lane 3 = MB-6, Lane 4 = MB-14, Lane 5 =MB-16, Lane 6 = MB-20, Lane 7 = MB-22, Lane 8 = MB-27, Lane 9 = MB-38, Lane 10 = MB-40, Lane 11 = MB-42, Lane 12 = MB-43, Lane 13 = MB-45, Lane 14 = MB-49, Lane 15 = MB-51.

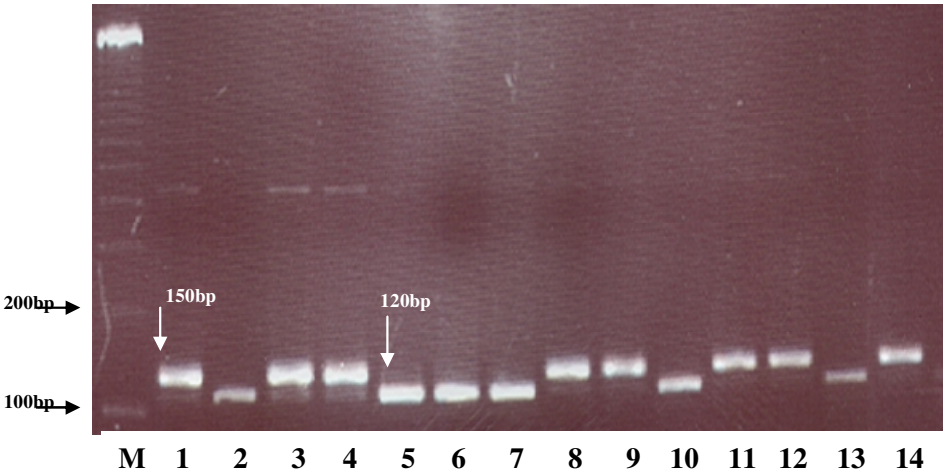

Figure 1b. Banding patterns showing the presence and absence of *Xa-4* gene in germplasm of rice amplified 150 bp and 120 bp size fragments respectively. Lane M = 100bp DNA ladder, Lane 1= IRBB-4, Lane 2 = IR-24, Lane 3 = MB-54, Lane 4 = MB-55, Lane 5 =MB-57, Lane 6 = MB-59, Lane 7 = MB-60, Lane 8 = MB-62, Lane 9 = MB-64, Lane 10 = MB-70, Lane 11 = MB-76, Lane 12 = MB-78, Lane 13 = MB-84, Lane 14 = MB-97.

Basmati 385 in the experiments could be due to the ineffectiveness of *Xa4* gene against the strains used in the study.

A study has been conducted on *Xoo* populations collected from different districts of Indian Punjab. They found high level of diversity in pathogen population collected from different parts of Punjab. They also found that BB resistance gene *xa8* and *Xa21* are effective against the prevalent isolates in Indian Punjab followed by *xa5* and *Xa7* (Sodhi et al., 2003). Indian Punjab is adjacent to the Basmati rice growing areas of Punjab in Pakistan; it could be possible that the same genes could be effective in Pakistan rice growing areas. However, studies on pathogen populations between countries and regions within countries have indicated that regionally defined pathogen populations are distinct, which could be attributed to the slow movement/dispersal of the pathogen or slow partitioning of host genotypes (Adhikari et al., 1995; Leach et

al., 1992; Nelson et al., 1994). Therefore, there is a need to identify other bacterial blight resistance genes in rice germplasm and Basmati breeding lines and also to check the effectiveness of identified bacterial blight resistance genes against the prevalent strain of *Xoo* in Pakistan. The knowledge of the effective resistance genes and the pathogen population structure would be helpful in deploying the suitable resistance genes in different rice growing areas.

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