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Full Length Research Paper

Molecular screening of Pakistani rice germplasm for *xa5* gene resistance to bacterial blight

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The narrow genetic base of cultivated rice cause vulnerability to bacterial blight (BB) because of an increased frequency of newly evolved pathotypes of greater virulence. Pyramiding of known resistance genes or scouting of new genes with a wider resistance spectrum, are the alternatives for breeding varieties with durable resistance. Molecular and conventional approaches were used to identify rice germplasm for the presence of *xa5*, a bacterial blight resistance gene. Polymerase chain reaction (PCR) with primers specific for *xa5* resistances gene were used in the study. During this polymorphic study, out of 60 rice lines, 31 were observed with *xa5* gene, while 29 showed the absence of *xa5* gene. Pakistani Basmati varieties were also surveyed. Out of the ten Pakistani Basmati varieties, Kashmir Basmati, Basmati Pak, Shahley Basmati and Basmati-622 had the *xa5* gene, while Basmati-385, Basmati-2000, Basmati-370, Basmati-198, Super Basmati and Dokri Basmati showed the absence of *xa5* gene. Identification of *xa5* gene in Pakistani rice germplasm will help in accelerating the breeding program including pyramiding of different disease resistant genes in basmati and other cultivated varieties.

Key words: Rice, germplasm, *xa5*, bacterial blight, near isogenic lines.

INTRODUCTION

Rice is one of the world's most important food crop; thus rice production and improvement are of interest to the Pakistan economy. Increasing the productivity of Pakistani rice cultivars through the development of resistant varieties would significantly increase the revenues generated by the crop. Bacterial blight (BB) is one of the most important diseases of rice in most rice growing countries due to its high epidemic potential and its destructiveness to high-yielding cultivars in both temperate and tropical regions, especially in Asia. The bacterial blight disease caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*) has been one of the major factors limiting rice production inAsia (Mew, 1987), resulting to an average of 20 to 30% yield loss. In some areas of Asia, rice yield losses are up to 50%. Agarwal et al. (2005) reported that in the Basmati rice, yield loss can reach up to 100%. The exploitation of host resistance has been shown to be the only reliable method to control the disease. More than 20 BB resistance genes, including 9 recessive genes, have been identified from cultivated rice and its wild relatives, or induced by mutagenesis (Lin et al., 1996; Nagato and Yoshimura, 1998; Zhang et al., 1998; Khush and Angeles, 1999; Chen et al., 2002; Lee et al., 2003; Yang

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Abbreviations: BB, Bacterial blight; **IRRI,** International Rice Research Institute; **IABGR,** Institute of Agricultural Biotechnology and Genetic Resources; **NARC,** National Agriculture Research Centre; **IRBB5,** Islamabad, Pakistan, Basmati varieties; **STS,** sequence tagged site; **PCR,** polymerase chain reaction; **NWFP,** North-West Frontier Province; **LL,** lesion length.

Table 1. Categorization of germplasms.

Infection (%)	Score	Host response
0	0	Highly resistant (HR)
>1 - 10	1	Resistant (R)
> 10 - 30	3	Moderately resistant (MR)
> 30 - 50	5	Moderately susceptible (MS)
> 50 - 75	7	Susceptible (S)
> 75 - 100	9	Highly susceptible (HS)

et al., 2003). Six genes are recessive: *xa5*, *xa8*, *xa13*, *xa24*, *xa26* and *xa28* that occur naturally and confer resistance; the other *xa*3, *xa15*, *xa19* and *xa20*, are created by mutagenesis and each confers a wide spec-trum of resistance to *Xoo* (Ogawa, 1996). 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, 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, 1989, Huang et al., 1997; Sanchez et al., 2000). In the present investigation, we were able to identify *xa5* gene in Pakistani rice varieties/indigenous land races.

MATERIALS AND METHODS

Plant materials

Thirty eight rice varieties/lines were obtained from the Institute of Agricultural Biotechnology and Genetic Resources (IABGR), National Agriculture Research Centre (NARC), Islamabad, Pakistan, Basmati varieties, IRBB5 (having *xa5* gene), IR-24 (without *xa*5 gene) and TN1 were obtained from International Rice Research Institute (IRRI), and 19 advance lines were developed at Hazara University. All these varieties/lines were grown in pots inside the greenhouse.

Isolation and multiplication of *Xoo*

Sixty diseased samples of rice leaves were collected from different areas of the North West Frontier Province (NWFP) and used for the isolation of *Xoo*.

Single cell culture

Single cell was taken with the help of sterilized inoculating wire loop from slimy yellowish bacterial colony developed around the infected samples and further streaked on nutrient agar plate in a zigzag manner. After streaking, plates were incubated at 25 to 26°C for 3 days.

Pathogenecity test/confirmation of pathogenic nature

All isolates were subjected to the pathogenecity test to confirm their pathogenic nature by injection infiltrations technique developed by Klemet (1963) and Klemet et al. (1964).

Inoculation of rice germplasm in glass house

Distilled water (5 ml) poured in each culture plates and bacterial colonies were suspended and the concentration of inoculums was adjusted to 10^8 cfu/ml. The suspension of all isolates was bulked in plastic bucket and shacked for uniformity. The plants were sprayed with water to create wet conditions which is favorable for disease development. Inoculation was done by cutting five leaves, approximately 5 cm from the tips of each line with scissor dipped in inoculums. On the basis of diseased data, these germplasms were categorized as resistant or susceptible using standard IRRI procedure (Table 1). After 14 days, diseased data were recorded to identify the degree of pathogenecity on 0 to 9 rating scale usig Standard Evaluation System IRRI (1996).

Sequence tagged site (STS) marker assisted confirmation of the presence of *xa*-5 gene in rice varieties/advance lines

Young leaves at seedlings stage were harvested for the isolation of genomic DNA. The DNA was extracted following the method of Dellaporta et al. (1983). The concentration of extracted genomic DNA was measured by a fluorometer. The DNA was diluted to 10 µg/µl, using sterilized distilled water and stored in microfuge tubes at 4°C for further use. Amplification of *xa5* linked fragment was carried out by using specific primers. Amplification reaction was carried out in 25 µl reaction volumes containing 50 ng genomic DNA, 1.0 µM each of the primer MP1 (5'ATT GTT ACG TTT GGT GGG GG 3') and MP2 (5'-GCC ATG GCG ACT GTC AGT CG -3'), 100 µM each of dATP, dCTP, dGTP, dTTP, 0.2 unit of Taq DNA polymerase, 1X Taq polymerase buffer and 2.5 mM $MgCl_2$. DNA amplification was performed in thermal cycle programmed as: 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 the final extension. Amplification product was resolved by electrophoresis on 1.5% of agarose gel. The amplified products were observed under Ultra Trans Illuminator after staining with ethidium bromide (10 µl/ml) and scored for the presence and absence of *xa5* linked DNA fragments.

Data analysis

The amplified fragment of all the rice genotypes/lines, basmati

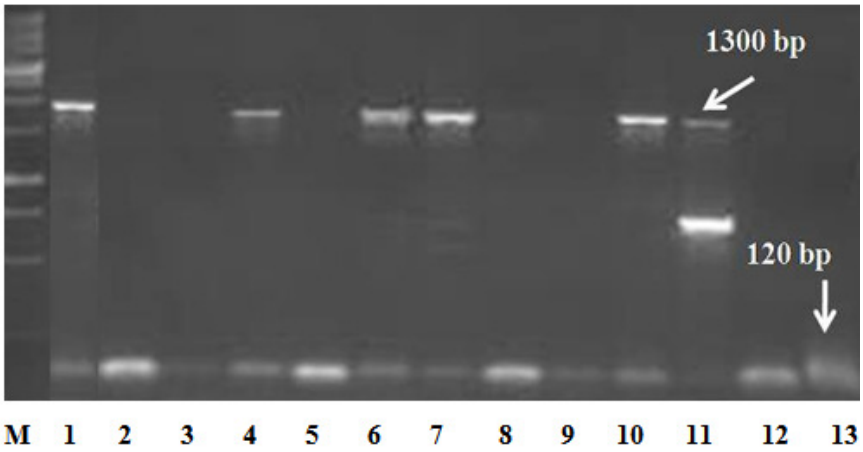

Figure 1. Banding patterns showing the presence and absence of *xa-5* gene in germplasm of rice amplified 1300 and 120 bp size fragments, respectively. Lane M = 1kb DNA ladder, Lane 1 = IRBB-5, Lane 2 = IR-24, Lane 3 = NIAB-IR9, Lane 4 = JP5, Lane 5 =PK177, Lane 6 = Jajai-77, Lane 7 = Swat-1, Lane 8 = Basmati-198, Lane 9 = Lateefy, Lane 10 = Basmati-pak, Lane 11 = MR10-3-10, Lane 12 = Mehlar-346 and Lane 13 = DR82.

breeding lines and the basmati varieties were observed and compared with IRBB5 and IR24 for the presence (+) and absence (-) of *xa5* gene.

RESULTS AND DISCUSSION

Attempts have been made to explore Pakistani rice germplasm for bacterial blight resistance gene *xa*5. Molecular and conventional approaches were used for confirming the presence of this gene. The molecular analysis of all the rice advance 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 IRBB5 (having *xa5* gene) or with that of the IR24 (without *xa5*) gene. The size of the band corresponding to IRBB5 is 1300 bp, whereas the band corresponding to IR24 is 120 bp in size (Figure 1). Blair and McCouch (1997) identified and synthesized this set of polymerase chain reaction (PCR) primers based on the sequence of a DNA marker tightly linked to rice BB resistance gene *xa5* for the survey of hybrid rice germplasm. Of the 27 resistance genes, STS markers are available for *xa5*, *xa13* and *Xa21* (Huang et al., 1997; Chunwongse et al., 1993).

During this polymorphic survey, out of 60 rice genotypes, 32 lines were observed with *xa5* gene, while 28 varieties/lines showed the absence of *xa5* gene. 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), it is time consuming and need artificial inoculation of all the lines with different pathotypes of the pathogen.

Of the 18 advance lines, 15 showed the presence of *xa5* gene. This implies that these advance elite lines are the source of *xa5* gene, which could be transferred to different varieties during the crossing and selection procedure. In this study, four cultivated Pakistani Basmati varieties, that is, Kashmir Basmati, Basmati Pak, Shahley Basmati and Basmati-C-622 showed the presence of *xa5* gene (Table 2). To reconfirm the presence of *xa*-5 gene, bacterial isolates were collected from North-West Frontier Province (NWFP) of Pakistan and inoculated at booting stage of the rice. The lesion length (LL) of tested varieties was measured 14 days after inoculation. The LL ranged from 8 to 66% (data not shown). The reactions of varieties carrying *xa*-5 gene ranged from moderately resistant to resistant. However, the varieties without *xa*-5 gene exhibited moderately resistant to susceptible reactions. Thirteen varieties carrying *xa*-5 gene showed moderately resistant reactions and 19 varieties exhibited resistant reactions. When inoculated with three isolates of *Xoo*, 14 varieties without *xa*-5 gene were moderately susceptible with shorter lesion length than susceptible check IR24 and lacked a sharp delineation of advancing lesion expression. When plants were inoculated with distinct BB isolates collected from NWFP, the consistent finding was that the lines which carry *xa-5* showed high degree of resistance over the lines lacking this gene. This shows that *xa*-5 contribute to resistance to *Xoo*. Similar results were also reported by Swamy et al. (2006) who evaluated bacterial blight resistance in transgenic. Both molecular and conventional approaches have been used by Ramalingam et al. (2001), Lee et al. (2003) and Kihupi et al*.* (2001) for the presence of *xa*5, *xa*13 and *Xa*21 in Chinese rice germplasm.

Marker assisted selection increases the efficiency of breeding program for selecting marker genotypes linked to target gene (Mohan et al., 1997). The identification of useful resistance genes through molecular analysis will support a gene deployment approach to manage the

Table 2. Reaction of rice varieties/advance lines with or without bacterial blight resistance gene *xa*-5 to *X. oryzae* pv *oryzae*.

Varieties/advance line	Target gene (*xa*-5)	Reaction to *Xoo*	Varieties/advance line	Target gene (*xa*-5)	Reaction to *Xoo*
Shadab	+	MR	Mehlar-346	-	MS
DR-92	+	R	Dokri Basmati	-	S
JP-5	+	MR	IR-6	+	R
NIAB-IR-9	-	MS	Basmati 2000	-	S
Fakhre Malakand	-	MS	Sathra	+	MR
DR-83	-	S	KS-282	+	MR
Shua 92	+	MR	Bas-385	_	S
Kashmir Basmati	+	R	DR-82	_	MS
Bas-C-622	+	R	Dilrosh-97	+	R
Mushkan	+	MR	MR5-2-2-1-7	+	MR
Pakhal	-	S	IRBB-5	+	MR
Bas-370	-	MS	IR-24	-	S
Swat-1	+	MR	MR5-2-2-1	+	MR
Jajai-77	+	R	MR10-3-7-2-5	+	MR
Sugdasi	-	MS	MR10-1-1	+	R
Bas-198	-	MS	MR10-8-3	+	MR
Sarshar	-	S	MR17-1-2-3	+	MR
IR-8	-	S	MR10-2-3	+	R
Sada Hayat	-	S	MR10-3-9-8	+	MR
PK-177	-	MS	MR10-3-10	+	R
TN1	-	MS	MR17-6-8-2	-	S
Nona Bokra	-	S	MR10-2-3-1	+	R
Pulman Sufaid	-	MS	MR10-12-5-4	-	S
Lateefy	-	MS	NPT-146	+	R
Khushboo-95	-	MS	NPT-89	-	S
Kangni 27	-	S	MR5-2-15	+	MR
Pakhal-2	-	S	MR5-2-7-1	+	MR
Super Basmati	-	S	RMR5-2-14	+	R
Bas-Pak	+	R	MRR17-10-12-1	+	MR
Shahley basmati	+	R	MRMR17-1-1-3-3S	+	R

R = Resistant; MR = moderately resistant; S = susceptible; MS = moderately susceptible; + = presence of *xa*-5; - = absence of *xa*-5.

disease using resistant cultivars. 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 (Abbasi et al., 2010). 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.

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