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Genotyping of rice germplasm for bacterial blight resistant gene (*Xa7*)

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Abstract

Rice (*Oryza sativa*) is one of most important cash crops in all over the world. Bacterial blight is a severe disease of rice caused by *Xanthomonas Oryza*. Abiotic control of this disease is expensive and cause environmental pollution. Therefore host plant resistance is one of the best ways to produce bacterial blight resistance in rice genotype. In present study bacterial blight resistance gene *Xa7* was investigated in important rice genotypes. Bacterial blight resistance gene *Xa7* was screened in 49 important rice genotypes by using specific primers linked to *Xa7*gene. The desire sequence (207 bp) of *Xa7* gene was amplified through a set of specific sequence tag site molecular markers through Polymerase Chain Reaction (PCR). Out of 49 genotypes, 23 showed the presence of *Xa-7* gene and these cultivars will be useful to control of this lethal disease.

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Introduction

The genus *Oryza* relates to the family Poaceae or gramanae. Oryza tribe has twelve genera. There are 22 species in the genus *Oryza* in which only *Oryza sativa* and *Oryza glaberrima* are cultivated and the rest are wild (Aggarwal *et al.,* 1997). These wild species have 2n=24 chromosomes representing AA, BB, CC, BBCC, CCDD, EE, FF, GG and HH genomes. But, unfortunately its yield and performance is highly affected by bacterial blight disease. Sakaguchi (1967) revealed that bacterial blight (BB) disease is most severe as compared to other diseases caused by bacterial pathogen. Bacterial blight is caused by *Xanthomonas oryza,* which is a severe disease. The destruction of the rice is due to bacterial blight disease especially Basmati rice in the Pakistan (Akhter *et al.,* 2004). First bacterial blight was reported in Japan during nineteenth century. Huang *et al.* (1997) described the factors which increased bacterial blight disease such as, high dosage of fertilizers, close spacing between plants and nonstop cropping for the improvement of crop. Bacterial blight is the most common in irrigated low land environment especially during the rainy months. The incidence percentage in Punjab ranges from 5-100% (Akhtar *et al.,* 2005).

The disease causes not only yield reduction but also lowers grain quality (Mew *et al.,* 1982). According to Mew *et al.* (1982) the control of bacterial blight by chemicals is difficult, although a few effective chemicals were reported however due to certain limitations like pollution, production costs; their use is restricted and with the passage of time the resistance to chemicals can developed by bacterium. Thus host plant resistance method is the most economical for controlling bacterial blight. For increasing the level of resistance in rice varieties, identification of the source of resistance is imperative and pre-requisite for more crop production. So far, more than 30 Bacterial blight resistance genes have been identified and designated in a series from *Xa1 to Xa32* (Chen *et al.,* 2004; Lee *et al.,* 2003; Lin *et al.,* 1996; Tan *et al.,* 2004). Yoshimura *et al.* (1995) cloned and characterized 7 bacterial blight resistance genes i.e., *Xa1, Xa3, Xa5, Xa13, Xa21, Xa26* and *Xa27*. Ullah *et al.* (2012) identified bacterial blight resistance genes *Xa4, Xa5, Xa7*, and *xa13* in 52 basmati landraces and five basmati cultivars using PCR markers. The *Xa7* gene was found to be the most prevalent among the cultivars and landraces. The gene pyramided are more resistant as contrast to variety having single resistant gene, the consequence of resistant gene can be incorporated and inherit gradually to next generation. Chen *et al.* (2008) reported that *Xa7* is dominant resistance gene against bacterial disease and give strong resistance against bacterial blight. Present study was undertaken to identify bacterial blight resistance (*Xa7*) gene in new local Pakistani and exotic rice germplasm through sequence tag sites marker.

Materials and methods

Plant materials

The research work was carried out at Plant Genetic Resources Institute (PGRI), National Agriculture Research Centre (NARC), Islamabad, Pakistan. Seeds of 49 rice accessions were acquired from Gene bank of PGRI (Table.1).

DNA extraction method

The healthy and mature seeds of 49 accessions were used for molecular analysis of the bacterial blight resistance gene. The DNA was extracted by following the method of Dellaporta *et al.* (1983). Three seeds from each variety were taken; seed coat was removed and placed in micro-centrifuge tube (1.5ml). 400µl of extraction buffer (200mM Tris.HCl (pH 8.0), 25mM EDTA, 200mM NaCl, 0.5% SDS), was added. To remove protein content 2µl of proteinase K (50µg) was added and incubated the solution at 37°C for 1 hour. Then the seed was grinded in the buffer with glass rod. After grinding, 400µl of 2% CTAB (100mM Tris-HCl (pH 8.0), 20mM EDTA (pH 8.0), 1.4M NaCl, 2% CTAB (w/v), 1% PVP solution was added. The solution was gently mixed using Chloroform: Isoamyl alcohol with 5% phenol (24:1:25). The debris was removed by centrifuging the mixture at 12,000rpm at 4°C for 10 minutes and the supernatant was then transferred into a new 1.5ml tube. For DNA

precipitation 2/3 volume of Isopropanol was added and incubated for 10 minutes at room temperature. After incubation the solution was centrifuged at 12,000 rpm for 5 minutes at room temperature. The supernatant was then removed. The DNA pellet washed with 70% ethanol (500µl), centrifuged at 12,000 rpm for 5 minutes at room temperature and poured off 70% ethanol. The DNA pellet was dried in vacuum dryer for 5 minutes and re-suspended in 50µl TE buffer. The RNA's were removed by adding 1µl of RNase (10 mg/ml).

Primer dilution (GDSSR02)

The primer in pellet form (56.6 nmol forward and 59.3 nmol reverse) was diluted to100 pmol/µl in TE buffer and kept under –24°C. For further use, primer was diluted to 20 pmol/ µl in sterile distilled water.

PCR amplification of Xa7

The DNA was diluted to 10µg/µL in TE buffer and stored at 4°C for further use. Amplification of *Xa-7* associated DNA portion of (207 bp) was carried out by the use of specific marker "GDSSR02" with forward sequence TGCCCACCGTCGAACTCGTGG and reverse AGCTAGCAATTCGCATGATTGC. Amplification of the reaction was carried out in 20µl reaction volumes containing 1µl of 50ng genomic DNA, 1µl (20 pmol) each of the primer, 0.4µl (10mM) of the dNTP's mix, 0.2µl (1unit) of Taq DNA polymerase, 2.0µl (10X) PCR buffer, 1.6µl (25mM) $MgCl_2$ and 12.8µl ddH_2O. The reaction mixture were then placed in thermal cycler and feed the DNA amplification programme specific to primer "GDSSR02", as shown in Table. 2. Amplified products were resolved in 2% agarose gel with 0.1µl/ml of Ethidium bromide (10µg/ml) for the visualization of DNA fragment. The amplified products were observed under UV trans-illuminator, the amplified fragment glows in the gel with the help of Ethidium bromide and were scored for the existence or lack of *Xa-7* associated DNA fragments.

Data analysis

The bands of all the gels were scored separately for the presence and absence of the *Xa7* bacterial blight resistance gene. The presence of the band indicated with (+) sign and absence of band indicated with (-) sign.

Results

In this study bacterial blight resistance gene *Xa7* was investigated in 49 accessions by using specific primer linked to *Xa7*. For this purpose PCR reaction was performed in 0.5ml tube with PCR cycles specific to primer match to *Xa7* gene.

Table 1. List of rice accessions.

Sr.#	Breeding Line	Accession	Sr.#	Breeding Line	Accession
1	HH2-11Y-11-Y3-D7	27773	26	RSP-2	27798
2	HH25-SAL-9Y3-Y1	27774	27	IRGC31699	27799
3	IRO-4L-191	27775	28	IRGC 26471	27800
4	IR-OSF101	27776	29	IRGC 31618	27801
5	R-SP-1	27777	30	IRGC 28986	27802
6	IR77080-B-34-1-1	27778	31	IRGC 115112	27803
7	IR795-97-56-1-2	27779	32	IRGC 31674	27804
8	IR80416-B-32-3	27780	33	IRGC 29151	27805
9	83140-B-11-B	27781	34	IRGC 28997	27806
10	IR83141-B-18-B	27782	35	IRGC 29014	27807
11	IR83140-B-28-B	27783	36	IRGC 31716	27808
12	IR-83140-B-32-B	27784	37	IRGC 27575	27809
13	IR-83140-B-36-B	27785	38	IRGC 27547	27810
14	IR-83142-B-6-B	27786	39	IRGC 26361	27811
15	IR-83142-B-7-B-B	27787	40	IRGC 26390	27812
16	IR-83142-B-8-B-B	27788	41	IRGC 20653	27813
17	IR-83142-B-9-B	27789	42	IRGC 29008	27814
18	IR-83142-B-79-B	27790	43	IRGC 25926	27815

19	IR-83142-B-49-B	27791	44	IRGC 33498	27816
20	IR-83142-B-21-B	27792	45	IRGC 27567	27817
21	IR-84678-25-5-B	27793	46	IRGC 31611	27818
22	IR-84677-21-1-B	27794	47	IR6	IR7
23	IR-84677-34-1-B	27795	48	JP5	JP6
24	IR-84675-7-3-2-B-B	27796	49	Super-Basmati	S. Basmati
25	IRRI-123	27797			

Table 2. PCR condition for primer GDSSR02.

Profile	Temperature	Time	No. of Cycles
Initial Strand Separation	94°C	4 minutes	1
Denaturation	94°C	30 second	
Annealing	58°C	45 second	35
Primer Extensión	72°C	1 minute	
Final Extensión	72°C	5 minutes	1
Soaking	4°C	Maintain	

To determine presence and absence of gene PCR product was analyzed in 2% high resolution agarose gel. Primer GDSSR02 tightly linked to dominant bacterial blight (BB) resistance gene *Xa7* and it amplified 207bp fragment. In this investigation 23 accessions showed the presence of *Xa7* gene, 207bp DNA fragment showing the presence of *Xa7* gene as shown in (Fig. 1-4). Twenty six accessions showed absence of *Xa-7* gene. The primer GDSSRO2 amplified specific band in, HH2-11Y-11-Y3-D7, HH25- SAL-9Y3-Y1, IRO-4L-191, IR-OSF101, R-SP-1, IR77080-B-34-1-1,IR80416-B-32-3, 83140-B-11-B,IR83141-B-18-B, IR83140-B-28-B, IR-83140-B-32-B, IR-83140-B-36-B, IR-83142-B-7-B-B, IR-84678-25-5-B, IR-84677-21-1-B, IRRI-123, RSP-2, IRGC 26471, IRGC 26361, IRGC 25926, IR6, JP5 and Super-Basmati (Table 3, Fig. 1-4).

Table 3. Presence and absence of *Xa7* gene in rice genotypes.

Sr.No.	Breeding Line	Accession	*Xa7* gene	Sr.No.	Breeding Line	Accession	*Xa7* gene
1	HH2-11Y-11-Y3-D7	27773	+	26	RSP-2	27798	+
2	HH25-SAL-9Y3-Y1	27774	+	27	IRGC31699	27799	-
3	IRO-4L-191	27775	+	28	IRGC 26471	27800	+
4	IR-OSF101	27776	+	29	IRGC 31618	27801	-
5	R-SP-1	27777	+	30	IRGC 28986	27802	-
6	IR77080-B-34-1-1	27778	+	31	IRGC 115112	27803	-
7	IR795-97-56-1-2	27779	-	32	IRGC 31674	27804	-
8	IR80416-B-32-3	27780	+	33	IRGC 29151	27805	-
9	83140-B-11-B	27781	+	34	IRGC 28997	27806	-
10	IR83141-B-18-B	27782	+	35	IRGC 29014	27807	-
11	IR83140-B-28-B	27783	+	36	IRGC 31716	27808	-
12	IR-83140-B-32-B	27784	+	37	IRGC 27575	27809	-
13	IR-83140-B-36-B	27785	+	38	IRGC 27547	27810	-
14	IR-83142-B-6-B	27786	-	39	IRGC 26361	27811	+
15	IR-83142-B-7-B-B	27787	+	40	IRGC 26390	27812	-
16	IR-83142-B-8-B-B	27788	-	41	IRGC 20653	27813	-
17	IR-83142-B-9-B	27789	-	42	IRGC 29008	27814	-

18	IR-83142-B-79-B	27790	-	43	IRGC 25926	27815	+
19	IR-83142-B-49-B	27791	-	44	IRGC 33498	27816	-
20	IR-83142-B-21-B	27792	-	45	IRGC 27567	27817	-
21	IR-84678-25-5-B	27793	+	46	IRGC 31611	27818	-
22	IR-84677-21-1-B	27794	+	47	IR6	IR7	+
23	IR-84677-34-1-B	27795	-	48	JP5	JP6	+
24	IR-84675-7-3-2-B-B	27796	-	49	Super-Basmati	S. Basmati	+
25	IRRI-123	27797	+				

Discussion

Pakistan is one of the best producers of rice crop. To increase its further export and local consumption more efforts is needed to improve its yield and production. This study was an attempt for the evaluation of 49 accessions through molecular markers and we successfully identified 23 different bacterial blight resistant rice genotypes. To analyze the performance of accessions grown under similar environmental condition, different strategies were used.

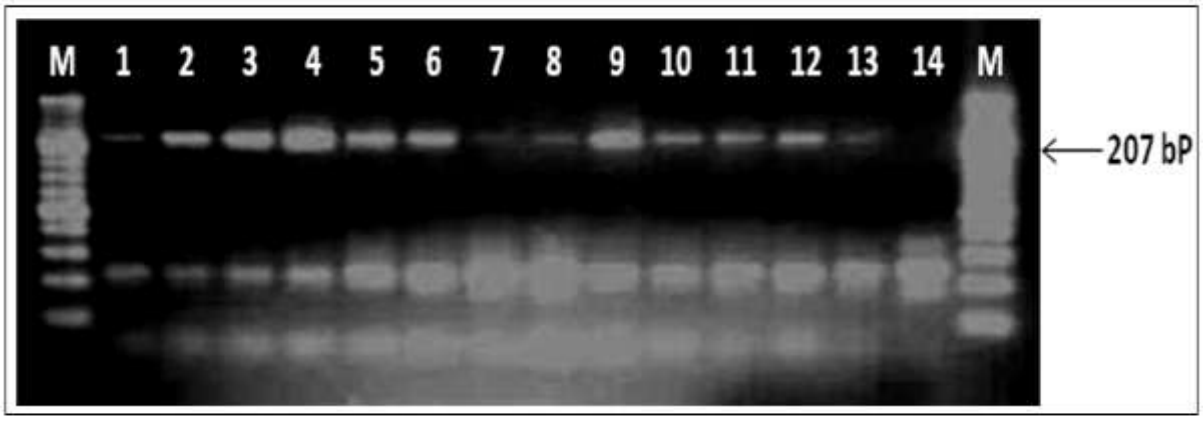

Fig. 1. PCR bands of 14 rice accessions, M=20bp DNA ruler, Lane1= HH2-11Y-11-Y3-D7, Lane2=HH25-SAL-9Y3-Y1, Lane3= IRO-4L-191, Lane4= IR-OSF101, Lane5= R-SP-1, Lane6= IR77080-B-34-1-1, Lane7= IR795-97-56-1-2, Lane8= IR80416-B-32-3,Lane9=83140-B-11-B, Lane10= IR83141-B-18-B, Lane11= IR83140-B-28-B, Lane12= IR-83140-B-32-B, Lane13= IR-83140-B-36-B, Lane14= IR-83142-B-6-B and Lane M=20bp DNA ruler.

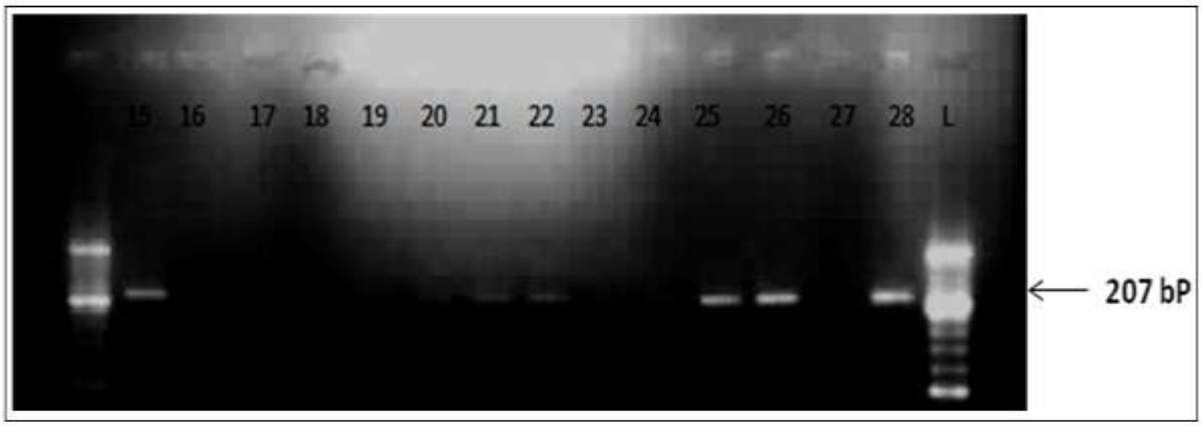

Fig. 2. PCR bands of 14 rice accessions, Lane L=20bp DNA ruler, Lane15= IR-83142-B-7-B-B, Lane16= IR-83142-B-8-B-B, Lane17= IR-83142-B-9-B, Lane18=IR-83142-B-79-B, Lane19= IR-83142-B-49-B, Lane20= IR-83142-B-21-B, Lane21= IR-84678-25-5-B, Lane22= IR-84677-21-1-B, Lane23= IR-84677-34-1-B, Lane24= IR-84675-7-3-2-B-B, Lane25= IRRI-123, Lane26= RSP-2, Lane27= IRGC31699, Lane28= IRGC 26471 and Lane L=20bp DNA ruler.

In present study, molecular evaluation of 49 rice accessions was performed for the presence and absence of *Xa7* BB resistance gene. Primer GDSSR02 was used for the amplification of desired bands. *Xa-7* lies between GDSSR02 at 0.21cM interval and produced 207 bp as reported by Chen *et al.* (2008). One more similar study was performed by Porter *et al.* (2003). They generated amplified fragment length polymorphic (AFLP) markers for X*a7* gene.

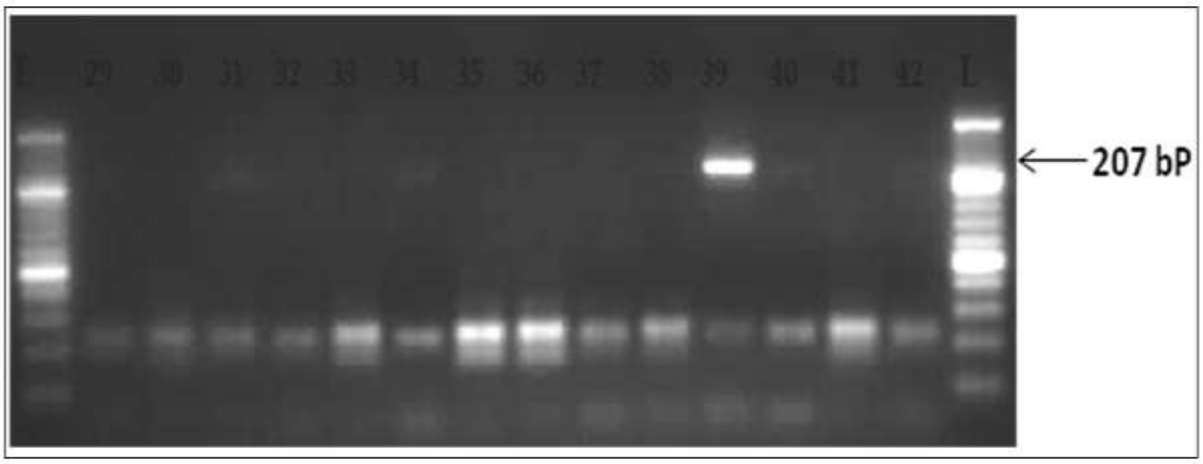

Fig. 3. PCR bands of 14 rice accessions, Lane L=20bp DNA ruler, Lane29= IRGC 31618, Lane30= IRGC 28986, Lane31= IRGC 115112, Lane32= IRGC 31674, Lane33= IRGC 29151, Lane34= IRGC 28997, Lane35= IRGC 29014, Lane36=IRGC 31716, Lane37= IRGC 27575, Lane38= IRGC 27547, Lane39= IRGC 26361, Lane40= IRGC 26390, Lane41= IRGC 20653, Lane42= IRGC 29008 and Lane L=20bp DNA ruler.

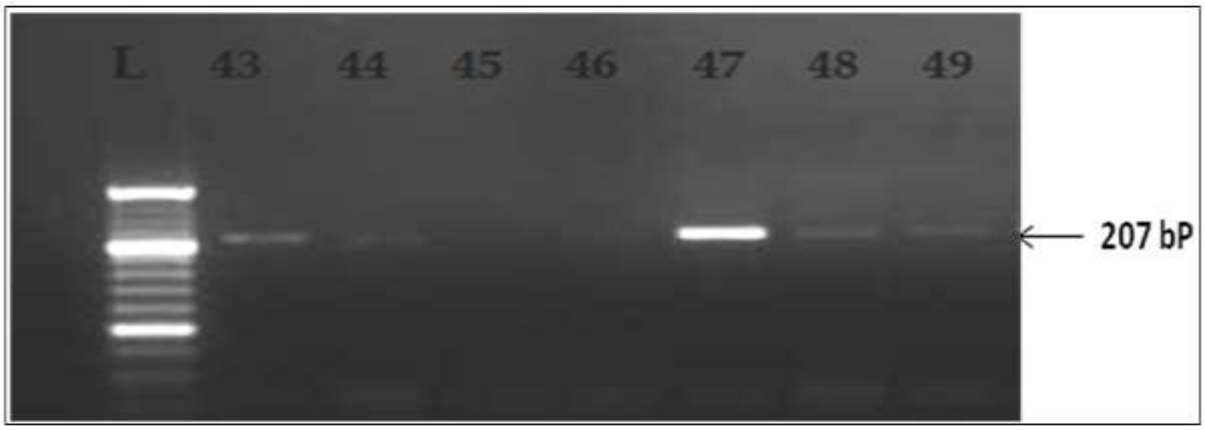

Fig. 4. PCR bands of 7 rice accessions, Lane L=20bp DNA ruler, Lane43= IRGC 25926, Lane44= IRGC 33498, Lane45= IRGC 27567, Lane46= IRGC 31611, Lane47= IR6, Lane48= JP5, Lane49= Super-Basmati and Lane L=20bp DNA ruler.

The F3 population of IR24-IRBB7 revealed that X*a7* gene lies distal to M1 at 107.3 cM and proximal to STS marker M2. Arif *et al.* (2008) performed similar research and identified *Xa4* bacterial blight resistant gene in rice germplasm/lines. The primers which were specific to *Xa-4* resistant gene were used for the detection of bacterial blight gene in 100 rice varieties. In this study 51 accessions showed the absence of *Xa4* gene. From 8 Pakistani basmati, 4 had *Xa4* gene. Amir *et al.* (2010) also conducted similar research, they found recessive *Xa-5* bacterial blight resistant gene in 88 Pakistani rice germplasm by using DNA marker produced 230bp piece linked to *Xa5* gene. Abbasi *et al.* (2011) reported the identification of Xa5 gene in advance lines of rice by STS marker. Ullah *et al.* (2012) identified bacterial blight resistance genes *Xa4, xa5, Xa7, a*nd *xa13* in 52 basmati landraces and five basmati cultivars using PCR markers.

Conclusion

Xanthomonas oryza cause bacterial leaf blight disease in rice that directly decreases its yield and other performance. In present study bacterial blight resistance gene *Xa7* was screened in 49 genotypes of rice. Out of 49 genotypes 23 showed the presence of gene with a set of specific target primers. The other 26 genotypes did not show the presence of gene in their genome, which is susceptible to this disease. These identified genotypes will be useful to control of this disease.

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