INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 6, No. 2, p. 318-324, 2015

RESEARCH PAPER OPEN ACCESS

Identification of bacterial blight resistance gene *Xa7* in rice *(Oryzae sativa L.)* through STS marker

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Key words: *Oryza sative*, Bacterial blight, STS markers.

http://dx.doi.org/10.12692/ijb/6.2.318-324 Article published on January 27, 2015

Abstract

Bacterial blight caused by *Xanthomonas Oryzae* pv. *Oryzae* (*Xoo*) is the most destructive disease of rice that limits rice yield in all major rice-growing regions of Pakistan, especially in irrigated lowland conditions. Since bacterial pathogen is difficult to manage, development of host plant resistance is the most effective mean to control this disease. In this investigation, a major gene (*Xa*-7) conferring broad spectrum resistance to various races of the pathogen has been identified in various varieties and advance lines of rice by STMS marker. Out of 74 rice varieties, 31 to 44 showed the presence of *Xa*-7. Identification of *Xa7* gene in rice could be utilized for increasing the level of resistance of existing rice varieties.

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Introduction

Rice (*Oryza sativa* L.) is one of the most important crop, which provides food for more than half of the world's population and is a source of calories for urban and rural inhabitants (Khush, 2005). Several biotic and abiotic threats had lowered the productivity and quality of rice in Pakistan. Among them bacterial blight is the most devastating disease that is caused by the Gram-negative proteobacterium *Xanthomonas oryzae* that lead to severe yield losses of up to 80% (Akhtar *et al* 2004, Srinivasan and Gnanamanickam, 2005), depending on the stage of the crop, cultivar susceptibility and the environmental conditions.

The most economical and environmentally safe measure for controlling BB is the exploitation of host plant resistance in combination with management practices. To reduce the losses of BB, several attempts have been made to identify and incorporate BB-resistance genes. To date, 34 genes (23 dominant and 11 recessive) conferring resistance against *X. oryzae* have been identified (Chen *et al*., 2011, Lin *et al*,1996; Nagato and Yoshimura 1998; Zhang *et al.* 1998; Khush and Angeles 1999; Chen *et al.* 2004; and Lee *et al*, 2003) largely in non-basmati rice. Several major resistance genes, including *Xa4*, *xa5*, *Xa7*, *xa13*, and *Xa21*, have been incorporated into rice cultivars (Perumalsamy *et al*., 2010). Recently, pyramiding of more than one major resistance gene has been confirmed to exhibit durable resistance against BB (Rajpurohit *et al*., 2010).

Conventional breeding methods are inefficient for gene determination, particularly in case of recessively inherited resistance genes, such as *xa5* and *xa13*. These limitations can be overcome by marker-assisted selection (MAS), which enables the evaluation of the expression of resistance gene (s) and allows for pyramiding of multiple resistance genes in susceptible varieties. Polymerase chain reaction (PCR)-based DNA markers for some of these genes have been identified: MP1 and MP2 for *Xa4* (Ma *et al*., 1999), RM122 for *xa5* (Chen *et al*., 1997), M5 for *Xa7* (Porter *et al*., 2003), and RG136 for *xa13* (Zhang *et al*., 1996). These markers have been employed to identify germplasm containing these genes (Blair and McCouch, 1997) and develop rice cultivars with single and multiple resistance genes (Perumalsamy *et al*., 2010; Rajpurohit *et al*., 2010).

In this study, we screened exotic, Pakistani land races and varieties for the status of the BB-resistance genes *Xa7* using STMS markers.

Materials and methods

Plant Material

The research work was carried out at National Agriculture Research Centre (NARC) Islamabad during 2010. Seeds of 74 rice varieties were obtained from Gene Bank of the Institute of Agricultural Biotechnology and Genetic Resources. Three healthy and mature seeds from each variety were used for molecular analysis.

DNA Isolation

DNA was isolated using a simplified miniscale procedure as reported by Dellaporta *et al* (2002) with some modification. A single piece of healthy young leaf was harvested and placed in a labeled 1.5 ml centrifuge tube on ice. The leaf sample was macerated using thick glass rod after adding 400 µl of extraction buffer (50 mM Tris-HCl, pH 8.0, 2.5 mM EDTA, 300 mM NaCl and 1% SDS). The sample was grounded until the buffer turned into green color. After grinding, another 400 µl of extraction buffer was added and mixed by pipetting. The contents were centrifuged at 12,000 rpm in micro centrifuge for 10 min. About 400 ul of lysate was extracted with 400 µl of chloroform. The top aqueous supernatant was transferred to another 1.5ml tube and DNA was precipitated with absolute ethanol and centrifuged for 3 min. The supernatant were used for PCR.

PCR Amplification of Xa7.

Amplification of *Xa7* was carried out by using tightly linked and co-segregated primers "GDSSR02" and "Rm20591" with forward sequence TGCCCACCGTCGAACTCGTGG and

reverse.AGCTAGCAATTCGCATGATTGC "F"TCGTCTGCGCGAATATTTAGAGAGG "R"ATCTGCATCGGAGTCAGCAACG respectively. Amplification of the reaction was carried out in 20µl reaction volumes containing 1µl of 50ng/µl genomic DNA, (20 pmol) of each primer, 10 mM of the dNTP's mix (Fermentas), one unit of taq DNA polymerase (Fermentas), PCR buffer (10X), 25 mM $Mgcl_2$ and double distilled water. After preparation of the reaction mixture, the tubes were placed in thermal cycler programmed as follows: For primer GDSSR02 an initial denaturation of 4 min at 94°C; 35 cycles of 94°C for 30 second (denaturation), 58°C for 45 second (annealing) and 72°C for one min (extension). For primer RM20591: initial denaturation of 4 min at 94°C, 35 cycles of 94°C for 30 sec, 55°C for 30 sec, 72°C for 1 min. and one additional cycle of 5 min. at 72°C was used for final extension. Amplification products were resolved by electrophoresis on 2% agarose gels. The amplified products were stained with ethidium bromide (10µg/µL) and observed under UV trans-illuminator. The bands were scored for the presence and absence of *Xa7* linked DNA fragment.

Results

In this investigation, 74 rice varieties were analyzed for the presence and absence of bacterial blight resistance gene *Xa7* by using PCR based techniques. Two primers, tightly linked to dominant bacterial blight resistant gene (*Xa7)* produced 207bp and 195bp fragments respectively. Both the primers: GDSSR02 and Rm20591 collectively exhibitted the presence of Xa7 in 17 rice varieties viz. Dilrosh-97, Dokri-Basmati, IR-9, Jajai-77, Kao-Dawk-Mali, Kinmaze, KS-282, KSK-133, Lateefy, Pakhal, PAU-201, PK-386, PK-177, Pusa-Basmati, Shau-92, Taichung-Native and Jasmine. GDSSR02 primer was tightly linked primer lies 0.28 cM from *Xa7* gene on rice chromosome 6 (Fig.1). Through this primer (Fig.2) 32 varieties showed the presence of the Xa7. While co-segregated primer RM20591 (Fig.3) amplified 195 bp fragment in 42 rice varieties (Table 1).

Table 1. Rice varieties used in present study to show the presence (+) and absence (-) of *Xa7* gene through GDSSR02 (M1) and RM20591 (M2).

S.No	Varieties	M1	M2	S.No	Varieties	M1	M2
1	IR-8	+	-	38	Mahlar-346	-	-
2	Azueena	-	-	39	Mehak	-	+
3	Basmati-2000	-	+	40	Mushkan	-	-
4	Basmati-370	-	-	41	Mutant-370	-	-
5	Basmati-385	-	+	42	NIAB-IR9	+	-
6	Basmati-Pak	-	+	43	Niaw-Hawn-Mali	+	-
7	Basmati-C622	-	+	44	Nippon bare	-	-
8	Basmati-198	-	+	45	Pakhal	+	+
9	Chini-Sakkor	-	+	46	Palman-sufaid	+	-
10	Dehradun-Basmati	-	+	47	PAU-201	+	+
11	Dilrosh-97	+	+	48	PK-386	+	+
12	Dokri-Basmati	+	+	49	PK-177	+	+
13	DR-82	+	-	50	Pokkoli	-	-
14	DR-83	+	-	51	Purple-marker	-	+
15	DR-92	+	-	52	Punjab-Basmati 1	-	+
16	Fakhre Malakand	+	-	53	Pusa-1121	-	+
17	IR-36	-	-	54	Pusa-Basmati	+	+
18	IR-6	+	-	55	Rachna-Basmati	-	+
19	Mehman 67	-	-	56	Ranbir-Basmati	-	+
20	IR-8	+	-	57	Sada-hayat	-	+
21	IR-9	+	+	58	Sarshar	+	-
22	Jajai-77	+	+	59	Sathra	-	-

23	Jhona-349	-	+	60	Shadab	-	+
24	JP-5	+	-	61	Shaheen-Basmati	-	+
25	Kagni-27	-	-	62	Shahkar	-	+
26	Kangni-XTorh	-	+	63	Shandar 2006	+	-
27	Kanwal-95	-	+	64	Shua-92	+	+
28	Kasalath	-	-	65	Sonahri-kangni	-	+
29	Kashmir-Basmati	-	+	66	Sonahri-Sugdasi	-	-
30	Khao-Dawk-Mali	+	+	67	Sugdasi-sadagulab	-	+
31	Khao-Jao-Haum	-	+	68	Sugdasi-Bengalo	-	+
32	Kharai-ganga	-	-	69	Sugdasi-Ratria	-	+
33	Khushboo-95	+	-	70	Super-Basmati	-	+
34	Kinmaze	+	+	71	Swat-1	+	-
35	KS-282	+	+	72	Swat-2	-	-
36	KSK-133	+	+	73	Taichung- Native	+	+
37	Lateefy	+	+	74	Jasmine-scented	+	+

Discussion

Host-pathogen interaction, determine the susceptibility or resistance reaction of the host plant. With the passage of time, the pathogen, develop resistance and crop become susceptible. The most effective approach to control the pathogenic effect of bacteria is to investigate new resistant host in local germplasm, wild species or mutants.

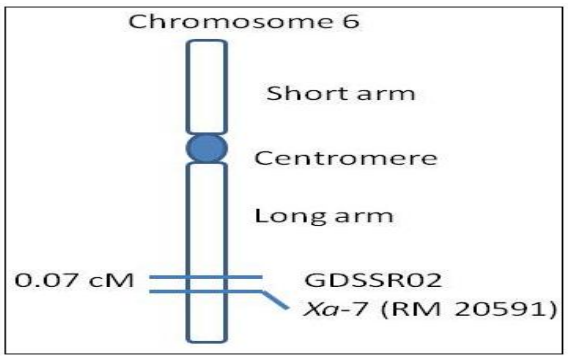

Fig. 1. Approximate position of *Xa*-7 on chromosome 6 of rice.

Xa-7 is a dominant gene that has been mapped on chromosome 6 with an interval of 0.07 cM between GDSSR02 and RM20591 and mediate resistance in adult stage of the plant. Recombination frequency between *Xa*7 and G1091 was 0.8%. and was 22.1cM away from marker S12715. Both *Xa7* and *Xa* 27 are dominant genes and have different resistance spectrum rending that both are not allels. *Xa*33(t) is closely linked with marker RM20590 which cosegregate with *Xa*7, however, the resistance behavior is different indicating that *xa*33 and *Xa*7 are not allels.

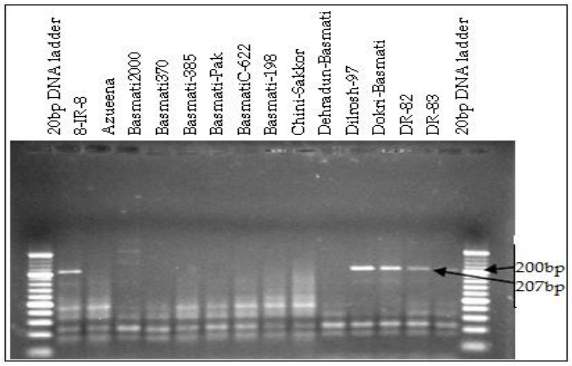

Fig. 2. Banding pattern of 14 rice varieties showed the presence and absence of the bands amplified by a tightly linked primer GDSSR02 to bacterial blight resistance gene *Xa7*.

This is the first attempt to explore Pakistani germplasm for *Xa* 7. Two markers closely linked to this gene were used in this investigation. The consistent finding was that among the 74 lines studied, 17 lines exhibited the presence of Xa7 by both primers. Although conventional approach for the identification of resistance genes have been used (Lee *et al*, 2003, Kihupi *et al* 2001) it was time consuming and need artificial inoculation with different pathotype and pathogen (Abbasi *et al*, 2010, Abbasi *et al*, 2011). (Arif *et al*, 2008) conducted the similar study for the identification of *Xa4* gene in Pakistani rice germplasm. Primers specific for *Xa4* resistances gene were used in the study to identify *Xa4* gene in 100 rice germplasm. We were used the same procedure and analyzed *Xa7* gene in rice varieties. (Huilan *et al*, 2002) identified bacterial blight resistance gene *Xa25* from a restorer line Minghui 63.

This gene conferred resistance to Philippine race 9 (PXO339) of *X. oryzae* pv. *oryzae* in both seedling and adult stages. It was mapped on chromosome 12 at 2.5 cM from a disease resistance gene homologous sequence 7.3 cM from a restriction fragment length polymorphism marker. In another study (Abbasi *et al*, 2010) produced 12 NILs; these lines were analyzed by a pair of primers linked to *Xa21* gene. Three lines were highly resistant to *Xa21* bacterial blight resistance gene.

Marker assisted selection approach is very effective for exploring the resistance in the germplasm. The knowledge of resistance and the prevailing pathogene population may be helpful in deploying suitable resistance gene in different rice growing areas. There is need to identify other BB resistance genes in rice varieties, land races, wild species and also to check the effectiveness of identified bacterial blight resistance genes against the prevalent strain of Xoo in Pakistan (Abbasi *et al*, 2010).

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