

## Detection of *xa13*- a Recessive Resistance Gene Against Bacterial Blight in Pakistani Rice Germplasm

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**Abstract:** Pakistan is a leading exporter of high quality fragrant rice in the world. However the confluence of narrow genetic base of host resistance in existing cultivars, the newly evolving and relatively more virulent strains of causal organism of bacterial blight (*Xanthomonas oryzae* pv *oryzae* (*Xoo*)) and suitable environmental conditions can potentially play havoc with the quality and quantity of rice crop. To achieve effective resistance to *Xoo*, in Pakistani rice cultivars, the breeder need to focus on how to pyramid the known resistance genes against this particular disease at the same time continuing to discover and characterize new genetic sources for broad based and lasting resistance. Molecular survey was conducted to identify advanced lines and some varieties for the presence of *xa13*, a bacterial blight resistance gene. A total of 52 rice lines and 6 varieties of rice were screened with polymerase chain reaction (PCR) using specific primers for *xa13* resistance gene. Our results showed that only 23 advanced lines and 4 varieties contained *xa13* gene. The discovery and characterization of *xa13* gene in indigenous germplasm will enable breeders to develop disease resistant cultivars of rice in relatively shorter time-frame.

**Key words:** Bacterial blight • Disease resistance • *xa13* gene • Rice

### INTRODUCTION

Rice is one of most the important food crop worldwide. Rice production and improvement are important to the economy of Pakistan because it is one of the staple foods in the daily life and a major export commodity. Increasing the productivity of Pakistani rice crop through the development of resistant varieties would significantly increase food security and the revenues generated through export. Bacterial blight caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*) is one of the most important diseases of rice in most of the rice producing countries of the world due to its high epidemic potential and effects on high-yielding cultivars in both temperate and tropical regions especially in Asia. The bacterial blight disease is one of the major limiting factors in rice production in Asia that result an average 20-30% yield loss [1]. In China, when the hybrid rice was attacked in the late seventies, the losses were reported to be as high as

50% in severely infected fields. Under certain conditions there was a likelihood of total crop failure necessitating re-planting [2]. Yield loss can reach up to 100% in Bastmati rice in extreme situations [3].

Host resistance has been shown to be the only effective remedy against this disease. So far 27 resistance genes, named *Xa*-1 to *Xa*-27 have been identified for resistance against various strains of *Xoo*. Fourteen of these genes have been successfully mapped to chromosomes 4 to 8, 11 and 12 [4- 8]. Out of 27 resistance genes conferring resistance to bacterial blight, sequence tagged site (STS) markers are available for *xa5*, *xa13* and *Xa21* [9, 10]. The lines in which resistance genes have been found against *X. oryzae* include cultivated rice, its wild cultivars and lines in which mutations have been artificially induced [5, 6, 7, 11- 14]. Among the genes that confer resistance over a wide spectrum to *X. oryzae*, six of the genes i.e. *xa5*, *xa8*, *xa13*, *xa24*, *xa26* and *xa28* occur naturally; and another four i.e. *xa3*, *xa15*, *xa19* and *xa20*,

are found in lines created by mutagenesis [15]. Successful breeding of resistance varieties owe a lot to prior identification and characterization of qualitative and quantitative resistance genes. The exploitation of resistance vested in such genes has shown complete resistance to pathogens in some modern cultivars [9, 16, 17]. *Xa4* which has conferred durable resistance in many rice cultivars has been mentioned as one of the most widely exploited resistance gene in many Asian rice breeding programs [18].

Resistance mediated by *xa13* is race-specific, e.g. (*xa13*-mediated resistance) has been defeated by some strains of *X. oryzae pv oryzae* [13, 19]. The exact mechanism of how *xa13* is defeated is unknown [20]. However, the use of indigenous lines as donor parents in hybridization with Basmati cultivars already containing genes *Xa4* and *Xa7* has been investigated for expediting the efforts to develop bacterial blight resistant rice [21]. Therefore, in this study, we have attempted to screen the available Pakistani rice germplasm lines and commercial varieties for the presence of a recessive gene *xa13* gene.

## MATERIALS AND METHODS

**Plant Materials:** Fifty two advanced lines and six varieties of rice were obtained from the Agricultural Research Center Mingora Swat Pakistan. In addition, IRBB59 (having *xa13* gene), IR-24 (without *xa13* gene) were obtained from International Rice Research Institute (IRRI) Philippines and used as a control in the PCR.

**DNA Isolation and PCR Amplification of *xa13*:** Young soft leaves of tested rice genotypes at seedlings stage were harvested for the isolation of genomic DNA. The DNA extraction was carried out using the protocol [22]. The quantification of genomic DNA was conducted through fluorometer. The quantified DNA was then diluted to 10 µg/µl, using sterilized distilled water and was stored in microfuge tubes at 4°C before further molecular use. Amplification of *xa13* linked fragment was carried out by using specific primers. Amplification reaction was carried out in 25 µl containing 50ng genomic DNA of rice, 1.0µM each of the primer F (TCCCAGAAAGCTACTACAGCA') and R (GCAGACTCCAGTTTGACTTC), 100µM each of dATP, dCTP, dGTP, dTTP, 0.2 unit of *Taq* DNA polymerase, 1X *Taq* polymerase buffer and 2.5 mM MgCl<sub>2</sub>. DNA

amplification was performed in thermal cycler programmed as an initial denaturation of 5 minutes at 94°C, 35 cycles of 94 °C for one minute (denaturation) 55 °C for 1 minute (annealing) and 72°C for 2 minutes (extension). One additional cycle of 10 minutes at 72°C was carried out for final extension. Amplification product was analyzed by gel electrophoresis using 1.5 % of agarose. The amplified products observed were under Ultra Trans Illuminator after staining with ethidium bromide (10 µl/ml) and scored for the presence and absence of *xa13* linked DNA fragments.

**Data Analysis:** The scored amplified fragments of all Basmati rice land races were detected and assessed against IRBB59 and IR24 for the presence (+) and absence (-) of *xa13* gene.

## RESEULTS AND DISCUSSION

Bacterial blight caused by *Xanthomonas oryzae pv. oryzae* (*Xoo*) is a significant disease of rice in Asia. Pakistan has a major share in growing and exporting rice however the crop is prone to bacterial blight [23, 24]. To raise the level of resistance to bacterial blight in rice germplasm, the availability of the source of resistance is imperative. In the present study, attempts have been made to explore the source host rice of resistance in indigenous germplasm for the presence or absence of bacterial blight resistance gene *xa13* for effective rice breeding strategies. The set of PCR primers used in the present study i.e. STS marker (RG136) was identified and synthesized based on the sequence of a DNA marker tightly linked to the rice bacterial blight (BB) resistance gene *xa13* for the survey of hybrid rice germplasm. Similary, bacterial blight resistance genes *xa-5*, *xa13* and *Xa21* in Chinese rice germplasm have been investigated [21, 25, 26].

DNA analysis of all the rice germplasm exhibited two different bands. The observed banding pattern of all the individual samples were either identical with that of the IRBB59 (having *xa13* gene) or with that of the IR24 (without *xa13*) gene. The sizes of the amplified products corresponding to that of IRBB59 and IR24 are 1500bp and 120bp, respectively (Fig.1,2,3,4,5). This polymorphic survey showed that out of 58 rice genotypes, 27 rice genotypes were observed with *xa13* gene while 31 were found without *xa13* (Table 1) while only four of the six varieties were found to contain *xa13* gene.

Table 1: Lines of rice germplasm studied for possible presence (+) and absence (-) of *xa13* gene

S. No	Genotype	<i>Xa13</i>	S. No	Genotype	<i>xa13</i>
01	IR-31787-41-2-2-3-3	-	30	CT.8665-1-16-8-1	-
02	IR-32307-107-3-2-2	-	31	IR-57313-106-2-3	-
03	IR-39357-133-3-2-2-2	+	32	IR-62127-55-1-2-2-3	-
04	IR-50	-	33	IR-62164-32-2-2-2-1	-
05	IR-25340-64-1-3-1S	+	34	NANJING-57161	-
06	Kas-Bas/DG.WG	+	35	NANGING-67022	-
07	IR-31837-11-2-2-1-2	+	36	RP-2633-30-4-7	-
08	ECIA.66-84-1-1-1	-	37	Y.R.L/L-202-6S-5S-1S-1S	-
09	RP.1669-1529-4254	+	38	Y.R.L/L-2026S-5S-1S-2S	-
10	RGP.1335/IR.21841-81-3-2-1S	+	39	BG-1639	-
11	RGP.1335/IR.21841-81-3-2-2S	-	40	DAK-83-99-27-3	+
12	IR.44592-1-3-2-4	-	41	J-P-5/L-202-7S-1S-2S	+
13	RP-2263-2556-2	+	42	PARC-89(NIMAT)	+
14	BR-601-3-3-2-4	-	43	Bas:385/L-202-5S-1S-2S-1S-1S	-
15	ECIA.67-SI-J1-5	-	44	J-P-5/L-202-7S-1S-1S-2S	+
16	RP-2235-114-84-21	+	45	J-P-5/L-202-7S-1S-1S-7S	+
17	IR-59606-119-3	+	46	HEXI-22	+
18	IR-62873-469-2-9	+	47	ZHONG-YU-3	+
19	IR-52350-81-3-1-2	+	48	ILLABONG	+
20	IR-56381-139-2-2	-	49	PR-26881-PJ-16-4B-78-5-1	+
21	MILYANG-55(SAM GANG BYEO).	+	50	YUNLEN-2	-
22	KAOH SIUNG SEN YU 338	-	51	IRI-384	-
23	RP-1670-1481-2205-1585	-	52	GZ-5830-63-1-2	+
24	IR-62871-73-2-3	-	53	FAKHAR-I-MALAKAND	+
25	IR-62871-175-1-10	-	54	J-P-5	+
26	IR-62871-439-1-3	-	55	DILROSH-97	+
27	IR-62871-469-1-2	+	56	SWAT-1	+
28	Lawangai/Bas:385//Bas:385-1S-2S	-	57	SWAT-2	-
29	HSINCHU-64	-	58	Basmati-385	+



Fig. 1: Amplified DNA banding patterns showing the presence and absence of *xa-13* gene in advanced lines.

Lane M = 1kb plus DNA ladder, 1a=IR 24, Lane 2a = IRBB59, Lane 1 = IR-31787-41-2-2-3-3, Lane 2 = IR-32307-107-3-2-2, Lane 3 = IR-39357-133-3-2-2-2, Lane 4 = IR-50, Lane 5 = IR-25340-64-1-3-1S, Lane 6 = Kas-Bas/DG.WG, Lane 7 = IR-31837-11-2-2-1-2, Lane 8 = ECIA-66-84-1-1-1, Lane 9 = RP-1669-1529-4254, Lane 10 = RGP-1335/IR21841-81-3-2-1S, Lane 11 = RGP-1335/IR-21841-81-3-2-2S, Lane 12 = IR-44592-1-3-2-4, Lane 13 = RP-2263-2556-2, Lane 14 = BR-601-3-3-2-4.



Fig. 2: Amplified DNA banding patterns showing the presence and absence of *xa-13* gene in advanced lines.

Lane M = 1kb plus DNA ladder, Lane 1a=IR 24, Lane 2a = IRBB59, Lane 15 = ECIA-67-SI-JI-5, Lane 16 = RP-2235-114-84-21, Lane 17 = IR-59606-119-3, Lane 18 = IR-62873-469-2-9, Lane 19 = IR-52350-81-3-1-2, Lane 20 = IR-56381-139-2-2, Lane 21 = MILYANG-55(SAM GANGBYEO), Lane 22=KAOH SIUNG SEN YU 338, Lane 23=RP-1670-1481-2205-1585, Lane 24=IR-62871-73-2-3, Lane 25 = IR-62871-175-1-10, Lane 26 = IR-62871-439-1-3, Lane 27 = IR-62871-469-1-2, Lane 28 = Lawangai/Bas:385//Bas:385-1S-2S.



Fig. 3: Amplified DNA banding patterns showing the presence and absence of *xa-13* gene in advance lines.

Lane M = 1kb plus DNA ladder, Lane 1a=IR 24, Lane 2a = IRBB59, Lane 29 = HSINCHU-64, Lane 30 = CT.8665-1-16-8-1, Lane 31 = IR-57313-106-2-3, Lane 32= IR-62127-55-1-2-2-3, Lane 33 = IR-62164-32-2-2-2-1, Lane 34 = NANJING-57161, Lane 35 = NANJING-67022, Lane 36 = RP-2633-30-4-7, Lane 37 = Y.R.L/L-202-6S-5S-1S-1S, Lane 38 = Y.R.L/L-2026S-5S-1S-2S, Lane 39 = BG-1639, Lane 40 = DAK-83-99-27-3, Lane 41 = J-P-5/L-202-7S-1S-2S, Lane 42 = PARC-89(NIMAT),.



Fig. 4: Amplified DNA banding patterns showing the presence and absence of *xa-13* gene in advance lines.

Lane M = 1kb plus DNA ladder, Lane 1a=IR 24, Lane 2a = IRBB59, Lane 43 = Bas:385/L-202-5S-1S-2S-1S-1S, Lane 44 = J-P-5/L-202-7S-1S-1S-2S, Lane 45 = J-P-5/L-202-7S-1S-1S-7S-, Lane 46 = HEXI-22, Lane 47 = ZHONG-YU-3, Lane 48 = ILLABONG, Lane 49 = RP-26881-PJ-16-4B-78-5-1, Lane 50 = YUNLEN-2, Lane 51 = IRI-384, Lane 52 = GZ-5830-63-1-2, Lane 53 = FAKHAR-I-MALAKAND, Lane 54 = J-P-5, Lane 55 = DILROSH-97, Lane 56 = SWAT-1.

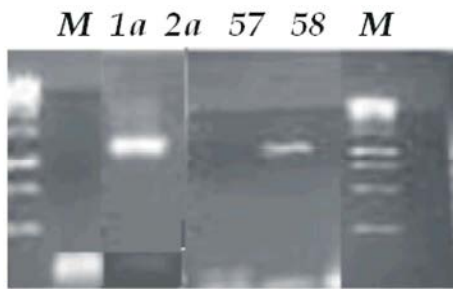


Fig. 5: Amplified DNA banding patterns showing the presence and absence of *xa-13* gene in advanced lines. Lane M = 1kb plus DNA ladder, Lane 1a=IR 24, Lane 2a = IRBB59 Lane 57 = SWAT-2, Lane 58 = Basmati-385.

Previous research on *Xanthomonas oryzae pv oryzae* pathosystem has yielded significant advancements in the understanding of molecular basis of interaction [27, 28]. However, continuous evolution of pathogenic races, breakdown of resistance keeps occurring in many improved varieties. Incorporation of resistance gene is very difficult using conventional methods of breeding due to epistasis or masking effect of other genes [1, 18]. The identification of useful resistance genes through molecular analysis will support a gene deployment approach to managing the disease using resistant cultivars. The present study implies that Basmati land races may be the origin of *xa13* gene, which could introgressed into other genetic backgrounds with hybridization and selection [21]. A detailed profile of the resistance genes available and the prevailing pathogenic strains would enable breeding of area-specific rice cultivars. Therefore, detailed characterization of resistance sources, whether qualitative or quantitative, available in indigenous germplasm and a thorough knowledge of *Xoo* strains prevalent in the area are necessary for countering bacterial blight in Pakistan.

Conventional approach for identifying resistance genes in rice germplasm, though still useful [13, 29], is laborious and time consuming as artificial inoculation of the lines to be tested is required with different pathotypes. The use of landraces containing recessive genes *xa5* and *xa13* as donor parents in hybridization with Basmati cultivars already containing genes *Xa4* and *Xa7* can be useful for developing bacterial blight resistant rice [21].

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