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Identification of bacterial leaf blight resistance genes in wild rice of eastern India

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Abstract: An experiment was conducted during the 2013 monsoon season to screen 35 wild rice accessions against the *BX043* strain of *Xanthomonas oryzae* pv. *oryzae* and identify the presence of bacterial blight resistance genes *Xa21*, *xa13*, *xa5*, *Xa4*, and *Xa2*. Among the accessions the area under the disease progress curve ranged from 174.88 (NKSWR-65) to 680.54 (NKSWR-34) compared with resistant controls RP bio-226 (93.92), CRMAS 2231-37 (097.28), CRMAS 2232-71 (098.58), and Tetep (178.62) and susceptible control PB-1 (1065.56). On the basis of disease severity 11 accessions showed moderate resistance, 21 were moderately susceptible, and 3 accessions showed susceptible response to the *BX043* strain of *Xanthomonas oryzae* pv. *oryzae*, while none of the accessions were found to be resistant. The genetic frequency of the 5 resistance genes varied from 00.00% to 45.71%. The accession NKSWR-25 harbored 3 resistance genes, *xa5*, *Xa4*, and *Xa2*, while accessions NKSWR-16, NKSWR-32, NKSWR-36, NKSWR-41, NKSWR-42, NKSWR-53, NKSWR-64, NKSWR-97, and NKSWR-99 each possessed 2 resistance genes of those 3 (*xa5*, *Xa4*, and *Xa2*). Therefore, these accessions could be used for the transfer of specific bacterial leaf blight resistance genes into well-adapted high-yielding rice cultivars.

Key words: Bacterial leaf blight, disease severity, SSR markers, resistance, wild rice

1. Introduction

Rice (*Oryza sativa* L.) is one of the oldest domesticated crops, which provides food for more than half of the world's population and constitutes a major source of calories for urban and rural inhabitants (Khush, 2005). Unfortunately, its production is constrained by a considerable number of diseases of fungal, bacterial, and viral origin. Bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is one of the most devastating diseases affecting entire rice acreages and causes severe yield losses of up to 80% depending on the stage of the crop, cultivar susceptibility, and environmental conditions (Srinivasan and Gnanamanickam, 2005). The genus *Oryza* consists of 22 wild and 2 cultivated species, *Oryza sativa* and *Oryza glaberrima*, belonging to the family Poaceae. Wild species of rice are reservoirs of many useful genes but a vast majority of these genes remain untapped, because it is often difficult to identify and transfer these genes into cultivated rice. Exploitation of host plant resistance is considered the most effective, economical, and environmentally safe measure for controlling BLB in combination with management practices. To tackle this problem, several attempts have been made to identify and characterize BLB resistance genes. To date, 34 genes (23 dominant and 11 recessive) have been identified that confer resistance to various strains of *Xoo* (Chen et al., 2011). Major resistance genes, including *Xa4*, *xa5*, *Xa7*, *xa13*, and *Xa21*, have been incorporated into rice cultivars in order to develop new resistant varieties (Perumalsamy et al., 2010). However, cultivars containing a single major resistance gene proved susceptible due to pathogen mutation. Recently, pyramiding of more than one major resistance gene has been proven able to deliver durable resistance against *Xoo* (Rajpurohit et al., 2010).

Conventional breeding tools are inefficient for gene pyramiding, particularly in the case of recessively inherited resistance genes such as *xa5* and *xa13*. These limitations can be addressed by marker-assisted selection (MAS), which enables the evaluation and expression of resistance genes and allows for pyramiding of multiple resistance genes in a desirable genetic background. Polymerase chain reaction (PCR)-based DNA markers for some of these genes have been identified and employed to identify germplasm containing these genes (Blair and McCouch, 1997) and to develop rice cultivars with single and multiple resistance genes (Perumalsamy et al., 2010; Rajpurohit et al., 2010). Thus, this study was carried out to identify the resistance genes *Xa21*, *xa13*, *xa5*, *Xa4*, and *xa2* in the wild rice so that efforts can be utilized to develop BLB-resistant rice cultivars through pyramiding approaches using MAS.

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2. Materials and methods

2.1. Plant material and experimental design

The experimental materials comprised 35 wild rice accessions of the Eastern Indo-Gangetic Region of India along with resistant (RP bio-226, CRMAS 2231-37, CRMAS 2232-71, and Tetep) and susceptible (Pusa Basmati-1) controls received from the Networking Project of the National Research Centre on Plant Biotechnology, New Delhi, India. Details of the collection sites of the wild rice accessions were presented in an earlier report by Chouhan et al. (2014). Single seedlings of 21 days old were transplanted with spacing of 20 × 15 cm apart in a plot size of 3.0 × 1.5 m in randomized block design with three replications at the Agricultural Research Farm, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during the 2013 monsoon season. Recommended practices were followed to raise a healthy crop.

2.2. Strain revival and pathogenicity test

The culture of *Xoo* (strain *BX043*, wild type) was obtained from the Directorate of Rice Research, Hyderabad, India, subcultured on peptone sucrose agar medium (distilled water: 1 L, sucrose: 20 g, peptone: 5 g, K_2HPO_4: 0.5 g, $MgSO_4.7H_2O$: 0.25 g, agar: 15 g), and maintained at pH 7.2–7.4 (Fahy and Persley, 1983). For pathogenicity testing, a clipping method was used to inoculate the rice plants with the *BX043* wild-type strain of *Xoo*. The test was conducted on fully developed leaves when rice plants were 45 days old after transplanting. The top 2.5–7.6 cm of completely developed leaves was clipped off one by one with sterilized scissors dipped in a bacterial suspension containing 10^8 cfu/mL.

2.3. Observations recorded and statistical analysis

Following inoculation, the plants were observed after every 24-h time interval to note the appearance of disease symptoms. The disease severity was recorded at 14, 21, and 28 days after inoculation from 10 randomly selected plants and 5 leaves per plant from each replication in each accession. On the basis of disease severity, all the accessions were classified as highly resistant/immune (0%), resistant (>1%–10%), moderately resistant (>10%–30%), moderately susceptible (>30%–50%), susceptible (>50%–75%), or highly susceptible (>75%–100%) by using a disease index of 0–9 (IRRI, 1996). The disease lesion length was measured with the scale from one end to another end covering the whole infected region of the leaf. Percent disease incidence was calculated with the help of the formula given by Gnanamanickam et al. (1999) and the area under the disease progress curve (AUDPC) by that given by Madden et al. (2007).

2.4. DNA extraction

Young leaves were collected from 35 transplanted wild rice accessions at 3 weeks old during the 2013 monsoon season. From each accession, 40 mg of leaves was placed in 1.2-mL collection microtubes (QIAGEN TissueLyser II, QIAGEN, USA), and in each microtube 3-mm tungsten beads were dispensed by a bead dispenser. Tubes were kept at –80 °C for 4 h. Tissues were disrupted and homogenized by the QIAGEN TissueLyser to a fine powder at a frequency of 30 vibrations per second for 30 s. Fine powdered leaf samples were used for the isolation of genomic DNA using the CTAB (hexadecyl trimethyl ammonium bromide) method (Doyle and Doyle, 1987). The DNA was quantified spectrophotometrically (PerkinElmer, Singapore) by measuring A260/A280, and DNA quality was checked by electrophoresis in 0.8% agarose gel.

2.5. SSR analysis

Five previously reported STS and SSR markers synthesized by Eurofins Genomics (Bangalore, India) were used to analyze the status of BLB resistance genes (Table 1). Amplification was carried out in a reaction mixture of 15 µL containing 30 ng of genomic DNA, 1.5 mM PCR buffer (MBI Fermentas, USA), 400 µM dNTPs (MBI Fermentas), 1 U of Taq DNA polymerase (MBI Fermentas), and 0.4 µM primer using a thermal cycler (Mastercycler Gradient, Eppendorf). The thermal cycling program involved an initial denaturation at 94 °C for 4 min, followed by 34 cycles of denaturation at 94 °C for 45 s, annealing at 2 °C below the Tm of the respective primers for 30 s, and primer extension at 72 °C for 30 s, followed by a final extension at 72 °C for 8 min. The amplified PCR products with a 100-bp DNA marker ladder (MBI, Fermentas) were size-fractioned by electrophoresis in 2.5% agarose gel prepared in TAE buffer and visualized by staining with ethidium bromide (0.5 µg/mL) in a gel documentation system (Bio-Rad, USA).

3. Results and discussion

3.1. Phenotypic screening for bacterial leaf blight resistance

Thirty-five wild rice accessions along with resistant (RP bio-226, CRMAS 2231-37, CRMAS 2232-71, and Tetep) and susceptible (Pusa Basmati-1) controls were screened against the *BX043* wild-type strain of *Xoo* under epiphytotic conditions during the 2013 monsoon season. The wild-type BX043 strain of *Xoo* is the most aggressive and highly virulent, mostly used to screen *Oryza sativa* species in India that give diverse responses with the host (Goel et al., 2002). The results of phenotypic screening are presented in Table 2. On the basis of disease severity at 28 days after inoculation, 11 accessions showed moderate resistance, 21 were moderately susceptible, and 3 accessions were susceptible to the *BX043* wild-type strain of *Xoo*. None of the accessions were found resistant-only due to the aggressiveness and high virulence of the *BX043* wild-type strain of *Xoo*. Similarly, wide responses

Table 1. List of BLB resistance genes, STS and SSR markers, and varieties used as controls.

S.N.	Gene	Chromosome locus	Control variety	Linked marker	Linkage distance (cM)	Primer sequence	Reference
1	*Xa21*	11	RP bio-226 (Improved Samba Mahsuri); CRMAS 2231-37 and CRMAS 2232-71	pTA248 (STS)	0.0	F: AGACGCGGAAGGGTGGTTCCCGGA	Ronald et al. (1992)
						R: AGACGCGGTAATCGAAGATGAAA	
2	*xa13*	8		xa-13prom (SSR)	0.0	F: GGCCATGGCTCAGTGTTTAT	Singh et al. (2011)
						R: GAGCTCCAGCTCTCCAAATG	
3	*xa5*	5		RM-13 (SSR)	17.9	F: TCCAACATGGCAAGAGAGAG	McCouch et al. (1996)
						R: GGTGGCATTCGATTCCAG	
4	*Xa4*	11	CRMAS 2231-37 and CRMAS 2232-71	RM-224 (SSR)	1.0	F: ATCGATCGATCTTCACGAGG	Sun et al. (2003)
						R: TGCTATAAAAGGCATTCGGG	
5	*Xa2*	4	Tetep	RM-317 (SSR)	18.5	F: CATACTTACCAGTTCACCGCC	He et al. (2006)
						R: CTGGAGAGTGTCAGCTAGTTGA	

of genotypes against *Xoo* was observed earlier by various workers (Ram et al., 2011; Thimmegowda et al., 2011; Sharma and Pandey, 2012). The initial symptoms of BLB, including linear yellow to straw-colored stripes with wavy margins, generally on both edges of a leaf and rarely on one edge, were observed with variable intensities. These symptoms first appeared in accession NKSWR-34 5 days after inoculation, while accessions NKSWR-65, NKSWR-55, NKSWR-75, and NKSWR-99 showed these symptoms after 8 days as compared to resistant controls (RP bio-226, CRMAS 2231-37, and CRMAS 2232-71), which showed symptoms 11 days after inoculation. Susceptible control PB-1 showed symptoms after 3 days. These findings are in agreement with an earlier report by Singh et al. (2013), who reported that the first symptoms appeared after 7 days in moderately susceptible rice germplasm. Among these wild rice accessions, NKSWR-65 and NKSWR-34 showed the lowest (14.73%) and highest (61.82%) disease severity, respectively. The AUDPC ranged from 174.88 (NKSWR-65) to 680.54 (NKSWR-34) compared with resistant controls RP bio-226 (93.92), CRMAS 2231-37 (097.28), CRMAS 2232-71 (098.58), and Tetep (178.62) and susceptible control PB-1 (1065.56). The moderately resistant accessions had AUDPC values ranging from 174.88 (NKSWR-65) to 358.95 (NKSWR-70), moderately susceptible from 329.77 (NKSWR-97) to 590.46 (NKSWR-25), and susceptible from 583.62 (NKSWR-41) to 680.54 (NKSWR-34). In the present study 19 accessions belonged to *Oryza rufipogon* and 16 to *Oryza nivara*. Eight *O. rufipogon* accessions were classified as moderately resistant, whereas only 3 *O. nivara* accessions were moderately resistant to the *BX043* strain of *Xoo*. Ten accessions of *O. rufipogon* and 11 of *O. nivara* were moderately susceptible. Therefore, in the present study, results showed that *O. rufipogon* accessions were more resistant than *O. nivara* accessions for BLB. These results are in conformity with the earlier findings of Shah et al. (2009).

3.2. Genotypic screening for BLB resistance

Thirty-five wild rice accessions were screened for the presence/absence of five BLB resistance genes, *Xa21*, *xa13*, *xa5*, *Xa4*, and *Xa2*, using PCR-based markers pTA248, xa-13prom, RM-13, RM-224, and RM-317, respectively linked to these genes. The resistant (RP bio-226, CRMAS 2231-37, CRMAS 2232-71, and Tetep) and susceptible (Pusa Basmati-1) controls were included as gene differential lines. Estimation of PCR results for the BLB resistance genes were determined by visualization of amplicons near 982 bp, 498 bp, 139 bp, 160 bp, and 154 bp of positive fragments, respectively. The results of genotypic screening of the 35 wild rice accessions are presented in Table 2, and electrophoretic patterns of SSR markers RM-13, RM-224, and RM-317 for resistance genes *xa5*, *Xa4*, and *Xa2*, respectively, are shown in the Figure. During this polymorphic survey, out of the 35 wild rice accessions, no amplicons specific to *Xa21* or *xa13* alleles were detected, showing the absence of these two genes in all the wild rice accessions evaluated except resistant control RP bio-226. Twelve accessions along with RP bio-226 amplified 139-bp fragments, 9 accessions along with CRMAS 2231-37 and CRMAS 2232-71 amplified 160-bp fragments, and 16 accessions along with Tetep amplified 154-bp fragments, indicating the presence of *xa5*, *Xa4*, and *Xa2* genes, respectively. Similar results were also

Table 2. Phenotypic and genotypic screening for BLB resistance in 35 wild rice accessions.

S.N.	Accession number/variety	Species/subspecies	Disease severity (%)			AUDPC	Host response	BLB resistance genes				
			14 DAI ± SD	21 DAI ± SD	28 DAI ± SD			*Xa21*	*xa13*	*xa5*	*Xa4*	*Xa2*
Resistant control	RP bio-226 (C_1)	*O. sativa*	05.40 ± 1.54	06.60 ± 1.81	08.40 ± 2.33	094.50	R	+	+	+	-	-
	CRMAS 2231-37 (C_2)	*O. sativa*	05.60 ± 1.11	06.73 ± 1.33	08.73 ± 1.53	097.28	R	+	+	+	+	-
	CRMAS 2232-71 (C_3)	*O. sativa*	05.67 ± 1.03	06.97 ± 1.20	08.57 ± 1.63	098.58	R	+	+	+	+	-
	Tetep (C_4)	*O. sativa*	08.93 ± 1.50	13.57 ± 1.80	14.97 ± 2.43	178.62	MR	-	-	-	-	+
Susceptible control	PB-1 (SC)	*O. sativa*	62.43 ± 7.60	73.10 ± 8.62	81.43 ± 10.13	1015.23	HS	-	-	-	-	-
1	NKSWR-1	*O. nivara*	28.80 ± 4.11	32.47 ± 6.25	38.57 ± 6.65	463.05	MS	-	-	-	-	+
2	NKSWR-2	*O. nivara*	33.97 ± 4.04	36.63 ± 4.20	38.13 ± 4.46	508.78	MS	-	-	-	-	+
3	NKSWR-4	*O. rufipogon*	13.07 ± 2.05	28.07 ± 4.00	28.07 ± 4.00	340.43	MR	-	-	+	-	-
4	NKSWR-9	*O. nivara*	24.97 ± 3.71	35.13 ± 3.97	35.13 ± 3.97	456.28	MS	-	-	-	-	-
5	NKSWR-12	*O. rufipogon*	23.93 ± 3.95	25.40 ± 4.70	27.80 ± 5.13	358.87	MR	-	-	+	-	-
6	NKSWR-16	*O. nivara*	33.80 ± 5.30	40.87 ± 5.54	47.20 ± 6.01	569.57	MS	-	-	-	+	+
7	NKSWR-25	*O. nivara*	37.73 ± 4.45	42.80 ± 4.60	45.97 ± 4.85	592.55	MS	-	-	+	+	+
8	NKSWR-26	*O. nivara*	15.47 ± 3.11	18.80 ± 3.60	21.47 ± 4.11	260.87	MR	-	-	-	-	+
9	NKSWR-32	*O. rufipogon*	20.03 ± 2.65	29.30 ± 3.32	34.47 ± 3.56	395.85	MS	-	-	+	-	+
10	NKSWR-34	*O. rufipogon*	30.67 ± 2.97	51.10 ± 4.03	61.43 ± 4.27	680.05	S	-	-	-	+	-
11	NKSWR-35	*O. nivara*	20.37 ± 2.85	31.83 ± 3.35	40.50 ± 3.86	435.87	MS	-	-	+	-	-
12	NKSWR-36	*O. nivara*	34.80 ± 3.76	40.80 ± 4.76	43.87 ± 4.96	560.93	MS	-	-	+	-	+
13	NKSWR-37	*O. nivara*	20.83 ± 3.13	27.83 ± 4.62	36.70 ± 5.33	396.20	MS	-	-	-	+	-
14	NKSWR-41	*O. nivara*	31.33 ± 3.06	42.67 ± 4.71	50.67 ± 5.70	585.67	S	-	-	+	-	+
15	NKSWR-42	*O. rufipogon*	22.83 ± 2.21	25.17 ± 2.66	28.17 ± 3.22	354.67	MR	-	-	+	+	-
16	NKSWR-46	*O. rufipogon*	16.67 ± 2.29	28.67 ± 2.65	36.50 ± 3.50	386.75	MS	-	-	-	-	-
17	NKSWR-48	*O. rufipogon*	18.87 ± 2.18	26.40 ± 2.69	29.73 ± 3.15	354.90	MR	-	-	-	-	-
18	NKSWR-49	*O. rufipogon*	22.90 ± 3.72	32.33 ± 3.85	42.33 ± 4.25	454.65	MS	-	-	-	-	-
19	NKSWR-51	*O. rufipogon*	11.57 ± 2.70	20.10 ± 2.95	28.70 ± 3.11	281.63	MR	-	-	+	-	-
20	NKSWR-53	*O. rufipogon*	20.50 ± 3.15	29.83 ± 4.67	36.17 ± 5.57	407.17	MS	-	-	+	-	+
21	NKSWR-54	*O. rufipogon*	28.83 ± 3.41	33.50 ± 3.51	37.17 ± 3.94	465.50	MS	-	-	+	-	-
22	NKSWR-55	*O. rufipogon*	09.43 ± 1.89	16.20 ± 2.21	22.50 ± 2.69	225.17	MR	-	-	+	-	-
23	NKSWR-57	*O. rufipogon*	14.83 ± 3.25	20.70 ± 3.46	24.83 ± 3.75	283.73	MR	-	-	-	-	-
24	NKSWR-64	*O. rufipogon*	14.13 ± 3.30	20.47 ± 3.82	24.17 ± 4.25	277.32	MR	-	-	-	+	+
25	NKSWR-65	*O. rufipogon*	09.90 ± 2.00	12.90 ± 2.36	14.73 ± 2.57	176.52	MR	-	-	-	-	+
26	NKSWR-70	*O. rufipogon*	20.90 ± 3.15	25.73 ± 3.46	29.73 ± 4.45	357.35	MR	-	-	-	+	-
27	NKSWR-73	*O. nivara*	15.67 ± 3.11	29.90 ± 3.39	37.20 ± 3.86	394.33	MS	-	-	-	-	-
28	NKSWR-75	*O. nivara*	12.97 ± 2.35	18.70 ± 2.79	22.53 ± 3.07	255.15	MR	-	-	-	-	+
29	NKSWR-82	*O. rufipogon*	16.33 ± 3.01	31.33 ± 3.69	41.00 ± 4.09	420.00	MS	-	-	-	-	-
30	NKSWR-84	*O. nivara*	21.67 ± 3.13	31.67 ± 4.12	37.23 ± 4.75	427.82	MS	-	-	-	-	+
31	NKSWR-85	*O. nivara*	29.30 ± 4.56	40.97 ± 5.73	53.83 ± 6.92	577.73	S	-	-	-	-	+
32	NKSWR-86	*O. rufipogon*	24.23 ± 2.36	29.90 ± 2.85	37.73 ± 3.41	426.18	MS	-	-	-	-	-
33	NKSWR-97	*O. nivara*	15.50 ± 3.90	24.17 ± 4.36	30.60 ± 5.05	330.52	MS	-	-	-	+	+
34	NKSWR-98	*O. rufipogon*	10.50 ± 2.29	24.17 ± 3.75	35.50 ± 4.27	330.17	MS	-	-	-	-	-
35	NKSWR-99	*O. nivara*	13.60 ± 3.26	18.13 ± 3.55	20.67 ± 4.20	246.87	MR	-	-	-	+	+
Frequency (%)								00.00	00.00	34.29	25.71	45.71
Approx. size (bp)								982	498	139	160	154

The rice BLB resistance gene was scored as the presence (+) or absence (-) of amplicons linked to five allele-specific SSR markers.
DAI: Days after inoculation, SD: standard deviation, AUDPC: area under disease progress curve, R: resistant, MR: moderately resistant, MS: moderately susceptible, S: susceptible, HS: highly susceptible, BLB: bacterial leaf blight.

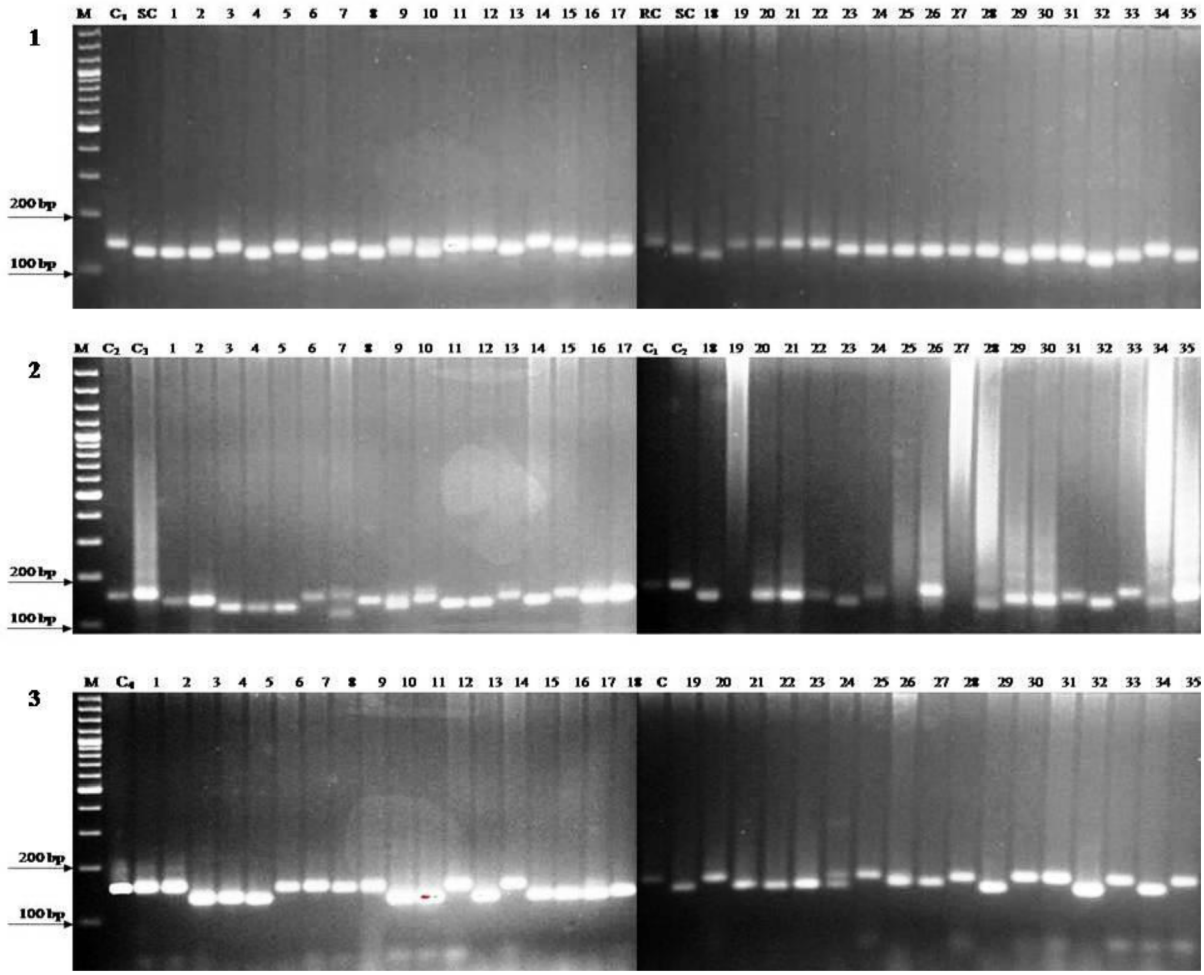

Figure. Agarose gel electrophoretic pattern of 35 wild rice accessions generated by using SSR markers (1) RM-13, (2) RM-224, and (3) RM-317, where M is 100-bp DNA size marker, C is resistant control variety, SC is susceptible control variety, and numbers 1–35 represent wild rice accessions as described in Table 2.

reported by Singh et al. (2012) in 42 landraces of rice; their results showed that 29 landraces carried an *Xa4*-specific allele whereas none of the landraces had *Xa21* or *xa13* genes. The resistance gene *Xa21* was isolated using map-based cloning strategies and it encodes proteins bearing nucleotide-binding sites and leucine-rich repeats. This gene was absent in all the accessions, which indicated that it was introgressed from *Oryza longistaminata*, a wild relative of the cultivated species *Oryza sativa* (Khush and Angeles, 1999). The resistance gene *xa13*, originally derived from landrace BJ1 from the Indian subcontinent, is effective individually as well as in combination with *Xa21*, *xa5*, *Xa4*, and *Xa2* against many pathotypes of *Xoo* (Lore et al., 2011). However, it is significant that the *xa13* gene was not detected in any of the wild rice accessions of this study. The present findings are in agreement with the reports of Davierwala et al. (2001), who surveyed rice genotypes popularly used in Indian breeding programs using markers closely linked to *xa5*, *xa13*. and *Xa21*. They reported that 8 lines carried *xa5*, only 2 had *Xa21*, and none of them carried *xa13*. The resistance gene *xa5* has been positionally cloned and encodes the gamma subunit of transcription factor IIA. Sequencing of transcription factor IIA in resistant and susceptible isolines revealed two nucleotide substitutions resulting in an amino acid change between resistant and susceptible cultivars. The identification and characterization of major genes for qualitative resistance and polygenic factors controlling quantitative resistance have contributed a great deal to success in breeding resistant cultivars. Many of these identified genes have been incorporated into modern rice varieties and exhibited complete resistance against the pathogens (Sanchez et al., 2000).

Among the wild rice accessions, 25.71% possessed the *Xa4* gene. The *Xa4* gene alone was not effective against many of the *Xoo* pathotypes evaluated from Punjab (Lore et al., 2011), and the broad-spectrum resistance observed here may be due to the presence of one or more additional genes or modifiers in most of the accessions. The *Xa4* gene is one of the most widely exploited resistance genes in many Asian rice breeding programs and it conferred durable resistance in many commercial rice cultivars (Mew et al., 1992). The pyramided lines with *Xa4* and other resistance genes showed a wider spectrum and a higher level of resistance than the lines with a single resistance gene (Huang et al., 1997). This calls for further detailed genetic analysis of the presence of novel BLB resistance genes and their tagging and cloning in wild rice accessions. This information can be gainfully utilized to supplement the BLB-resistant gene pool available in India. Wild rice accession NKSWR-25 harbored three resistance genes, *xa5*, *Xa4*, and *Xa2*, while accessions NKSWR-16, NKSWR-32, NKSWR-36, NKSWR-41, NKSWR-42, NKSWR-53, NKSWR-64, NKSWR-97, and NKSWR-99 each possessed two of those three genes. Sixteen wild rice accessions possessed only one gene of *xa5*, *Xa4*, and *Xa2*, while 9 accessions did not possess any resistance genes. Similarly, Ullah et al. (2012) reported that out of 52, only 10 basmati rice landraces had multiple resistance genes. These results also showed that the analysis of the distribution of resistance genes in wild rice accessions can direct BLB resistance breeding programs. The consistent results showed with the selected SSR markers for respective genes were highly reliable and make them the markers of choice for molecular screening of BLB-resistant genes among the rice accessions.

In conclusion, the presence of *xa5*, *Xa4*, and *Xa2* genes in wild rice accessions is lacking in modern cultivars. The use of these accessions as donor parents in hybridization with modern cultivars, which contain the genes *xa5*, *Xa4*, and *Xa2*, will expedite efforts to develop BLB-resistant cultivars through MAS-based pyramiding approaches without compromising yield and grain quality.

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