Identification and Fine-Mapping of *Xa33*, a Novel Gene for Resistance to *Xanthomonas oryzae* pv. *oryzae*

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ABSTRACT

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Broadening of the genetic base for identification and transfer of genes for resistance to insect pests and diseases from wild relatives of rice is an important strategy in resistance breeding programs across the world. An accession of *Oryza nivara*, International Rice Germplasm Collection (IRGC) accession number 105710, was identified to exhibit high level and broad-spectrum resistance to *Xanthomonas oryzae* pv. *oryzae*. In order to study the genetics of resistance and to tag and map the resistance gene or genes present in IRGC 105710, it was crossed with the bacterial blight (BB)-susceptible varieties 'TN1' and 'Samba Mahsuri' (SM) and then backcrossed to generate backcross mapping populations. Analysis of these populations and their progeny testing revealed that a single dominant gene controls resistance in IRGC 105710. The BC_1F_2 population derived from the cross IRGC 105710/TN1//TN1 was screened with a set of 72 polymorphic simple-sequence repeat (SSR) markers distributed across the rice genome and the resistance gene was coarse mapped on chromosome 7 between the SSR markers RM5711 and RM6728 at a genetic distance of 17.0 and 19.3 centimorgans (cM), respectively. After analysis involving 49 SSR markers located between the genomic interval spanned by RM5711 and RM6728, and BC_2F_2 population consisting of 2,011 individuals derived from the cross IRGC 105710/TN1//TN1, the gene was fine mapped between two SSR markers (RMWR7.1 and RMWR7.6) located at a genetic distance of 0.9 and 1.2 cM, respectively, from the gene and flanking it. The linkage distances were validated in a BC_1F_2 mapping population derived from the cross IRGC 105710/SM//2 × SM. The BB resistance gene present in the *O. nivara* accession was identified to be novel based on its unique map location on chromosome 7 and wider spectrum of BB resistance; this gene has been named *Xa33*. The genomic region between the two closely flanking SSR markers was in silico analyzed for putatively expressed candidate genes. In total, eight genes were identified in the region and a putative gene encoding serine-threonine kinase appears to be a candidate for the *Xa33* gene.

Additional keywords: disease resistance, molecular mapping.

Rice (*Oryza sativa* L.) is the principal food crop of the world. It plays a pivotal role in the Indian economy, being the staple food for two-thirds of the population. To meet the increasing food demands for the future, concerted efforts are required to increase rice productivity and minimize production losses due to pest and disease. Among the biotic stresses afflicting rice, bacterial blight (BB) is an important disease that causes significant yield reduction worldwide. The disease, in its severe form, is known to cause yield losses of 74 to 81% (30). Host plant resistance is considered the most economical and ecofriendly strategy for achieving disease resistance and yield stability. However, widespread cultivation of some of the resistant varieties carrying a single BB resistance gene has led to evolution of virulent populations of the pathogen that are capable of overcoming resistance (27). Widening of the genetic base through identification of novel resistance genes from wild relatives of rice, their deployment in breeding programs, and pyramiding two or more effective resistance genes are some of the approaches envisioned for developing rice cultivars with durable resistance to *Xanthomonas oryzae* pv. *oryzae*. Many of the wild species of *Oryza* such as *O. longistaminata*, *O. rufipogon*, *O. minuta,* and *O. nivara* have been reported to be resistant to BB (2). Five BB resistance genes—*Xa21*, *Xa23*, *Xa27*, *Xa29t*, and *Xa30t*—have so far has been identified from *O. longistaminata*, *O. rufipogon, O. minuta, O. officinalis*, and *O. nivara,* respectively (4,9,28,33,38,39). Though many of the wild species of *Oryza* have been observed to possess resistance to *X. oryzae* pv. *oryzae*, the nature and genetics of resistance to *X. oryzae* pv. *oryzae* in these accessions have not been analyzed thus far. Addressing these concerns, the present study was designed to (i) screen the accessions of wild species of *Oryza* and identify donors possessing resistance to multiple strains of *X. oryzae* pv. *oryzae*, (ii) study the inheritance of resistance to *X. oryzae* pv. *oryzae* in the selected donors, (iii) tag and map the novel genes' resistance to *X. oryzae* pv. *oryzae* using molecular markers, and (iv) perform in silico analysis of putative candidate genes.

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*The *e*-Xtra logo stands for "electronic extra" and indicates that the online version contains four supplementary tables.

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MATERIALS AND METHODS

Plant materials. In all, 105 wild rice accessions belonging to different species of *Oryza* such as *O. nivara* (93 accessions), *O. longistaminata* (5 accessions), *O. glaberrima* (2 accessions), and *O. officinalis* (5 accessions) maintained at the Directorate of Rice Research, Hyderabad (Supplementary Table 1) were evaluated for

resistance to *X. oryzae* pv. *oryzae*. An accession of *O. nivara* (International Rice Germplasm Collection [IRGC] 105710), which was found to exhibit high level of resistance to multiple BB strains in repeated tests, was crossed with BB susceptible varieties such as 'TN1' and 'Samba Mahsuri' (BPT5204) to develop mapping populations. The first set of mapping populations consisted of 435 progeny-tested BC_1F_2 plants derived from the cross IRGC 105710/TN1//TN1, which were developed by selfing a single BB-resistant BC_1F_1 plant. A second set of mapping populations, consisting of 2,011 progeny-tested BC_2F_2 plants derived from the above-mentioned cross, was used for fine-mapping studies. A third set of mapping populations, consisting of 410 progeny-tested BC_1F_2 plants derived from the cross IRGC 105710/Samba Mahsuri//Samba Mahsuri by selfing a BB resistant BC_1F_1 plant, was used for validation of linkage distances of the markers identified in the earlier mapping population. For allelism tests for the BB resistance gene present in IRGC 105710, crosses were made between a homozygous resistant BC_1F_2 plant derived from the cross IRGC 105710/TN1//TN1 with near-isogenic lines (NILs) of IR 24 possessing known single BB resistance genes, IRBB1 (*Xa1*), IRBB4 (*Xa4*), IRBB7 (*Xa7*), IRBB8 (*xa8*), and IRBB21 (*Xa21*) sourced from the All India Coordinated Rice Improvement Project. The F_2 populations were developed for the above-mentioned crosses and screened for BB resistance in order to identify whether the gene derived from the *O. nivara* accession is allelic to any one of the known genes for resistance to *X. oryzae* pv. *oryzae*.

Screening with *X. oryzae* pv. *oryzae* strains. Seven different virulent strains of *X. oryzae* pv. *oryzae*, isolated from various BB hot-spot locations of India and maintained at the Directorate of Rice Research, Hyderabad, were used for screening the plant materials. The details of these isolates are given in Supplementary Table 2. The *X. oryzae* pv. *oryzae* strains were grown as described by Sundaram et al. (32) and the plants were screened for BB resistance by the leaf-clip inoculation method (15). To evaluate the BB reaction of the rice plants, they were raised in cement pots in the glasshouse. Each accession was grown in three pots. Observations were recorded on five inoculated leaves in each pot. The mean lesion length along with standard error was then calculated. The mapping populations were grown in plastic trays in the glasshouse and the plants at the tillering stage were clip inoculated with BB pathogen. Observations were recorded on five inoculated leaves of each plant by recording the lesion length and the plants were categorized as resistant (average lesion length <4 cm) or susceptible (average lesion length >4 cm) as per Shanti et al. (25). TN1 and Samba Mahsuri were used as susceptible checks, while different NILs of IR 24 (IRBB lines) possessing the resistant genes *Xa4*, *xa5*, *xa13*, and *Xa21* and their combination were used as resistant checks.

A progeny test of the BC_1F_2 and BC_2F_2 mapping populations was carried out in three replications by screening a set of 40 to 50 BC_1F_3 or BC_2F_3 seedlings from each BC_1F_2 or BC_2F_2 plant along with the susceptible (TN1) and resistant (IRGC 105710) checks using strain DX-066 of the pathogen. A BC_1F_2 and BC_2F_2 line was scored as homozygous resistant, if all its progeny exhibited resistant reaction. On the other hand, if all the progeny were susceptible, it was scored as homozygous susceptible. Those BC_1F_2 and BC_2F_2 lines whose progeny exhibited segregation with respect to lesion length were scored as heterozygous resistant after statistically conforming goodness of fit for the 3:1 resistant/susceptible (R/S) ratio (expected for segregation of a single dominant resistance gene) through χ^2 tests. Both resistant and susceptible checks were included while screening the mapping population (7).

Molecular mapping analysis using simple-sequence repeat markers. DNA from the parents and mapping populations was isolated using the method of Dellaporta et al. (6). To map the BB resistance gene or genes from IRGC 105710, 238 simple-sequence repeat (SSR) markers (data not shown) uniformly spread across the 12 linkage groups of rice (at approximately one marker per 2 Mb of physical distance on each chromosome) were screened for parental polymorphism. Their map locations, primer sequences, and other details were identified based on information available online at http://www.gramene.org. The polymorphism between the donor parent IRGC 105710 and recipient parents TN1 and Samba Mahsuri was analyzed following the polymerase chain reaction (PCR) conditions described by Chen et al. (5). The PCR products were resolved on 4% Seakem LE agarose gels (Lonza, Walkersville, MD) in a 0.5× Tris-borate-EDTA buffer, stained with ethidium bromide (0.5 µg/ml), and photographed under UV light (24). Bulked segregant analysis (BSA) (21) was carried out by pooling equal quantities of DNA from 10 plants each of homozygous resistant and homozygous susceptible BC_1F_2 plants derived from the cross IRGC 105710/TN1//TN1 to generate resistant bulks (RB) and susceptible bulks (SB), respectively. DNA amplification of the resistant and susceptible parents and the RB and SB was done by using the parental polymorphic SSR markers. Markers which displayed bulk-specific amplification were subjected for analysis in the entire mapping population. Fine mapping was carried out using a set of 49 SSR markers specific to chromosome 7. These markers were selected based on their relative physical positions on the rice genome near the two coarsely linked, flanking SSR markers RM5711 and RM6728. Of the 49 SSR markers selected, 39 were from the RM series and 10 SSR markers were specifically designed for the present study targeting the microsatellite repeats in the genomic region of interest (SSR markers RMWR7.1 to RMWR7.10). The details of these markers are given in Supplementary Table 3.

Marker-trait linkage analysis. Linkage analysis and map construction were performed using MAPMAKER/EXP, version 3 (18). The genotype data for the polymorphic markers were corroborated with the phenotypic data in relation to resistance or susceptibility of the individual BC_1F_2 or BC_2F_2 plants, which was deduced through progeny testing. Linkage groups were obtained using two-point analysis with a log-likelihood of odds (LOD) score of 4.0 and maximum recombination level of 0.3. This step was implemented by using the 'group' command. Linked markers within the linkage groups were ordered using multipoint analysis with 'compare', 'suggest subset', and 'try' commands. Best order of marker was then confirmed with the 'ripple' command, using a minimum LOD score of 4.0. Finally, the map distance was calculated using 'map' command. The map distances were converted into centimorgans using the Kosambi (16) function. Linkage maps were prepared for the different mapping populations separately.

Identification of putative candidate genes. The genomic sequence between the flanking SSR markers was downloaded from Japonica rice genome and analyzed using the online software FGENESH (http://www.softberry.com). All genes with clear open reading frames (ORFs) were identified and a putative function for these genes was annotated using BLAST-P utility (http://www.ncbi.nlm.nih.gov) to identify the putative function of each gene identified in the region of interest.

RESULTS

Identification of BB-resistant wild rice accessions and study of inheritance of BB resistance. Eighteen accessions of *O. nivara* were observed to be resistant to all seven virulent strains of *X. oryzae* pv. *oryzae*, with an average lesion length of 1.6 to 3.8 cm in repeated inoculation tests under controlled conditions. Inheritance of BB resistance in these wild rice accessions was studied by crossing them with the BB-susceptible varieties TN1 and Samba Mahsuri. Among the F_1s generated from the 18 crosses, 17 showed near-complete to partial spikelet sterility with very little or no F_2 seed set. The F_1s derived from the cross IRGC

105710/TN1 displayed partial spikelet fertility and some F_2 seed (≈130) could be collected from F_1s of this cross. However, the F_2s displayed pronounced segregation distortion when analyzed with a set of five SSR markers (data not shown) and, hence, could not be used for inheritance or gene mapping studies. The accession IRGC 105710 was highly resistant (average lesion length = 1.63 cm) and displayed a wider resistance spectrum when compared with the NILs possessing the major BB resistance genes such as *Xa4, xa5*, *xa13*, and *Xa21* (Supplementary Table 4). Because the F_1s of this cross were resistant, it was presumed that IRGC 105710 may possess one or more dominant resistance genes. The F_1s were backcrossed with the susceptible parents TN1 and Samba Mahsuri to obtain BC_1F_2 and BC_2F_2 mapping populations, respectively. The populations were screened with isolate DX-066 to study the inheritance of resistance to *X. oryzae* pv. *oryzae*. The BC_1F_2 mapping populations derived from the cross with TN1 and Samba Mahsuri, consisting of 435 and 410 plants, respectively, displayed a segregation ratio of 3R:1S (χ^2 = 1.41, $P > 0.23$; χ^2 = 0.42, $P > 0.47$). The BC_2F_2 population obtained from the cross IRGC 105710/TN1//2 × TN1, consisting of 2,011 plants, also displayed segregation in a ratio of 3R:1S (χ^2 = 1.25, $P > 0.263$), indicating the action of a single dominant gene controlling the resistance to *X. oryzae* pv. *oryzae* in IRGC 105710. In the allelism tests, the F_2s of the crosses with IRBB1, IRBB4, IRBB7, and IRBB21 segregated in a ratio of 15R:1S while the F_2s derived from IRBB8 cross segregated in a ratio of 13R:3S (Table 1), indicating that the gene conditioning resistance in IRGC 105710 is nonallelic to *Xa1*, *Xa4*, *Xa7*, *xa8*, and *Xa21*.

Mapping of the dominant gene for resistance to *X. oryzae* pv. *oryzae* in IRGC 105710 using SSR markers. Of 238 SSR markers tested, 72 were polymorphic between IRGC 105710 and TN1. The SSR markers on chromosomes 1, 2, 3, 4, 5, 6, 8, 9, 10, 11, and 12 did not show any bulk-specific amplification pattern but the marker RM5711 located on chromosome 7 showed bulk-specific amplification, indicating the possibility of the presence of the BB resistance gene on chromosome 7. When RM5711 was analyzed in individual BC_1F_2 plants, recombinants were observed and the marker mapped at 17.0 centimorgans (cM) from the gene. To identify markers closely linked to the gene, 12 additional polymorphic SSR markers in the vicinity of RM5711 (which is

TABLE 1. Results of allelism test for known bacterial blight (BB) resistance genes for *Xanthomonas oryzae* pv. *oryzae*

Sample	Cross details	Population size	Number of plants[a]		Segregation ratio	χ^2 value	*P* value
			R	S			
1	BC_1F_2 (Plant number 198)/IRBB1	250	231	19	15:1	0.78	0.67
2	BC_1F_2 (Plant number 198)/IRBB4	197	180	17	15:1	1.90	0.38
3	BC_1F_2 (Plant number 198)/IRBB7	220	201	19	15:1	2.13	0.34
4	BC_1F_2 (Plant number 198)/IRBB8	286	240	46	13:3	1.17	0.27
5	BC_1F_2 (Plant number 198)/IRBB21	265	241	23	15:1	2.65	0.26

[a] R = resistant and S = susceptible.

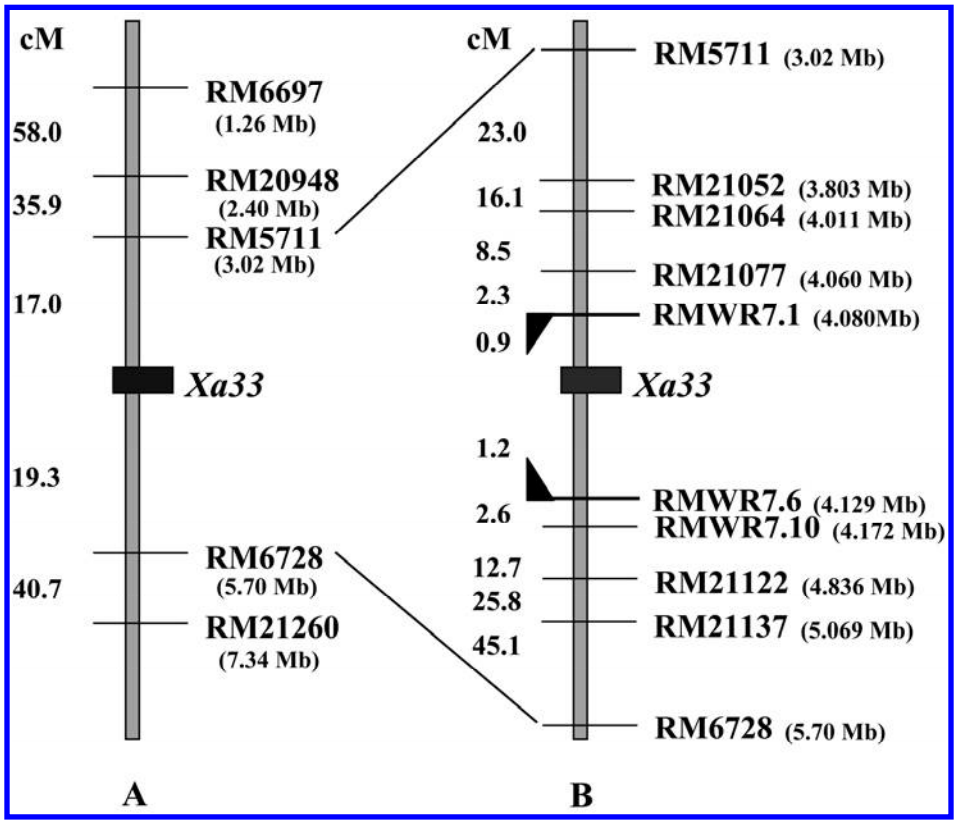

Fig. 1. Genetic linkage map of the genomic region in the vicinity of *Xa33* on chromosome7. **A,** The gene was initially coarse mapped between the simple-sequence repeat (SSR) markers RM5711 and RM6728 through analysis of 435 progeny-tested BC_1F_2 plants derived from the cross International Rice Germplasm Collection (IRGC) 105710/TN1//TN1. **B,** Later, it was fine mapped through segregation analysis of 2,011 progeny-tested BC_2F_2 plants derived from the cross IRGC 105710/TN/2 × TN1 utilizing a set of eight parental polymorphic SSR markers located between RM5711 and RM6728. RMWR7.1 and RMWR7.6 were found to be very close to the new dominant bacterial blight resistance gene (designated as *Xa33*) and flanking the gene at a genetic distance of 0.9 and 1.2 centimorgans, respectively. The linkage distances are given in centimorgans in the left side of chromosome arm, while the markers used for mapping/fine-mapping are given on the right side the chromosome arm. Mapping distances were calculated as Kosambi function utilizing the software Mapmaker 3.0.

located at 3.02 Mb on chromosome 7) were analyzed for their co-segregation with BB resistance, and a linkage map was constructed. The markers RM6697, RM20948, and RM5711 were mapped at a genetic distance of 58.0, 35.9, and 17.0 cM, respectively, from the gene on one side, while RM6728 and RM21260 were mapped on the other side of the gene at a genetic distance of 19.3 and 40.7 cM, respectively (Fig. 1).

Fine mapping of the dominant gene for resistance to *X. oryzae* pv. *oryzae* in IRGC 105710. Because none of the markers mentioned above are close enough for regular use in marker-assisted breeding or to accurately pinpoint the location of the resistance gene, a set of SSR markers located in the genomic interval spanned by the closest markers identified in the coarse mapping analysis (i.e., RM5711, located at 3.02 Mb, and RM6728, located at 5.70 Mb) were analyzed for their parental polymorphism. Among these, eight markers (RM21052, RM21064, RM21077, RM21122, RM21137, RMWR7.1, RMWR7.6, and RMWR7.10) were observed to be polymorphic and used for fine-mapping analysis in a set of 2,011 BC_2F_2 individuals derived from the cross IRGC 105710/TN1//2 × TN1. The SSR markers RM21052, RM21064, RM21077, and RMWR7.1 were mapped on one side of the gene at a genetic distance of 16.1, 8.5, 2.3, and 0.9 cM, respectively, while RMWR7.6, RMWR7.10, RM21122, and RM21137 were mapped on the other side of the gene at a genetic distance of 1.2, 2.6, 12.7, and 25.8 cM, respectively. The markers RMWR7.1 and RMWR7.6 were very close and were flanking the resistance gene in IRGC 105710 (which has been designated as *Xa33*). The segregation pattern of the closest flanking markers, RMWR7.1 and RMWR7.6, respectively, is shown in Figure 2A and B, while Figure 1 shows the genetic linkage map of chromosome 7 in the vicinity of *Xa33*.

Validation of the closely linked flanking markers in an alternate mapping population. The utility of the gene-linked SSR markers to predict trait phenotypes was validated in a progeny-tested BC_1F_2 population consisting of 410 plants derived from the cross IRGC 105710/Samba Mahsuri//Samba Mahsuri. The markers RM21077, RMWR7.1, RMWR7.6, RMWR7.10, and RM21122 exhibited polymorphism between the parents and were linked to *Xa33* at genetic distances which were similar to those observed in the BC_1F_2 mapping population derived from the cross IRGC 105710/TN1//TN1 (Table 2).

Analysis of amplification pattern of the closest, flanking SSR markers in rice germplasm. To examine whether the closely linked SSR markers RM21077, RMWR7.1, RMWR7.6, and RMWR7.10 could be utilized to introgress *Xa33* into elite rice cultivars, they were tested in eight selected, high-priority but

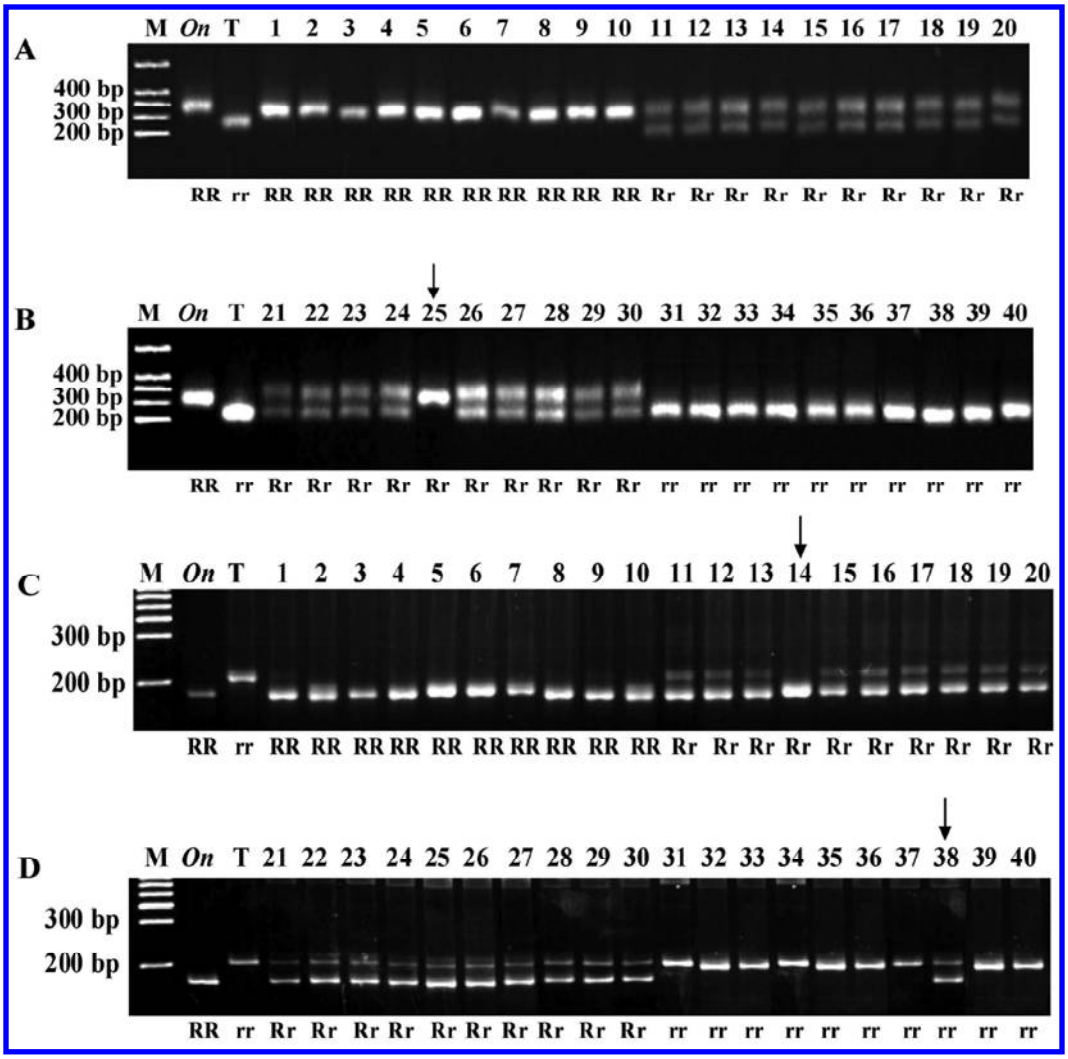

Fig. 2. Segregation pattern of the closely linked simple-sequence repeat (SSR) markers **A and B,** RMWR7.1 and **C and D,** RMWR7.6 in the BC_1F_2 population derived from the cross *Oryza nivara* (International Rice Germplasm Collection [IRGC] 105710)/TN1. *On* = *O. nivara*, T = 'TN1', M = molecular weight marker (100-bp ladder). Numbers along the top of each gel represent the number of the BC_1F_2 plant, while the genotype of the plant (as deduced from progeny testing at BC_1F_3) is represented in the bottom of the gel. RR and rr indicate homozygous resistant and homozygous susceptible BC_1F_2 plants, respectively, while Rr represents heterozygous plants. Arrow on the top of gels indicates recombinants. RMWR7.1 and RMWR 7.6 were observed to be closely linked to the new dominant bacterial blight resistance gene present in IRGC105710 (designated *Xa33*) at a genetic distance of 0.9 and 1.2 centimorgans (cM), respectively.

BB susceptible rice cultivars. All four SSR markers displayed polymorphism between the donor accession of *O. nivara* (i.e., IRGC 105710) and the BB-susceptible elite rice varieties (Fig. 3A and B), indicating that any of these markers could be used in breeding programs for targeted introgression of the resistance gene.

In silico analysis of the genomic region in the vicinity of *Xa33* for putatively expressed genes. Based on the fine mapping analysis, the SSR marker RMWR7.1 was located at a genetic distance of 0.9 cM on one side of the gene and RMWR7.6 was located on the other side of the gene at a genetic distance of 1.2 cM (Fig. 1). The intervening genomic region, consisting of ≈49 kb of sequence, was downloaded from the Japonica genome sequence database (http://rise.genomics.org.cn) and analyzed for the presence of putative genes using the software FGENESH (http://www.softberry.com). The functionality of the genes was analyzed using the BLAST-P tool (1), available online at http://www.ncbi.nlm.nih.gov. Eight genes were observed to be present in the genomic region flanked by RMWR7.1 and RMWR7.6. Of the eight genes identified in the region, one gene encoding serine threonine kinase, one encoding putative transposon protein and mutator protein, one encoding ribose-5-phosphate isomerase subfamily, one encoding domain of unknown function 295, one encoding DNA repair/transcription protein Mms-19-like protein, two encoding base inhibitor 1 (which belongs to a protein superfamily), and one encoding a hypothetical protein with no putative conserved domain were detected. The details of the genes along with their size in base pairs, amino acids, and their putative functions are given in Table 3.

TABLE 2. Genetic distance of the closely linked markers and *Xa33* in the two mapping populations

Sample	Closely linked markers	Genetic distance of the marker from *Xa33* (cM)	
		BC_1F_2[a]	BC_2F_2[b]
1	RM21077	2.3	2.5
2	RMWR7.1	0.9	1.0
3	RMWR7.6	1.2	1.2
4	RMWR7.10	2.6	2.5
5	RM21122	12.7	12.3

[a] As estimated in the BC_1F_2 population derived from the cross International Rice Germplasm Collection (IRGC) 05710/TN1//TN1.
[b] As estimated in the BC_2F_2 population derived from the cross IRGC105710/Samba Mahsuri//Samba Mahsuri population.

DISCUSSION

Development and deployment of BB-resistant cultivars is the only feasible strategy for managing BB, one of the most destructive diseases of rice. To date, 32 BB resistance genes have been identified from diverse sources (17). Even though the majority of the genes resistant to *X. oryzae* pv. *oryzae* have been identified from cultivated rice, a few, such as *Xa21, Xa23, Xa27, Xa29t*, and *Xa30t*, have been derived from wild species of *Oryza* (4,9,10, 19,28,33,38,39). Most of the BB resistance genes are dominant in nature (e.g., *Xa4*, *Xa7*, *Xa21, Xa23, Xa26,* and *Xa27*), while some are recessive (e.g., *xa5, xa8, xa13*, and so on). Most of the resistance genes have been introgressed into the background of the susceptible indica cultivar IR 24 and are available as NILs carrying single BB resistance genes (36). Some of the effective gene combinations such as *Xa21+xa13+xa5* and *Xa21+xa13* have been pyramided in the genetic background of elite rice varieties such as Samba Mahsuri (32), Triguna (31), and Pusa Basmati1 (14) in India. Despite these success stories, rice genotypes possessing widely deployed genes like *Xa4* (12) and *Xa21* (27) have been reported to be susceptible to some of the strains of the pathogen. This, indeed, is a matter of concern, and there is an emergent need to widen the repertoire of resistance genes available in order to enhance the spectrum and durability of resistance to *X. oryzae* pv. *oryzae*. In this regard, BB-resistant wild relatives of the genus *Oryza* can be tapped to identify novel genes for resistance to *X. oryzae* pv. *oryzae*.

In the present study, wild rice accessions maintained at the Directorate of Rice Research, Hyderabad were screened for resistance to *X. oryzae* pv. *oryzae* using a set of seven virulent strains of the BB pathogen. Even though few accessions of *O. nivara* were observed to display a broad-spectrum resistance, when they were crossed with TN1, the F_1s derived from all the resistant accessions except IRGC 105710 showed near-complete to partial spikelet sterility with very little or no F_2 seed set, precluding the possibility of using them for inheritance and gene mapping studies. Partial seed set was noticed in the F_1s derived from the cross IRGC 105710/TN1. Pronounced segregation distortion was noticed in the F_2s based on analysis with a few SSR markers (data not shown). The phenomenon of segregation distortion has been frequently reported in wide crosses in many crop species and is well documented in intraspecific and interspecific crosses in rice (11,20,35). The preferential transmission of gametes resulting in segregation distortion might be due to gamete elimination, pollen killer genes, and the phenomena of restriction in recombination in the hybrids derived from wide crosses (4).

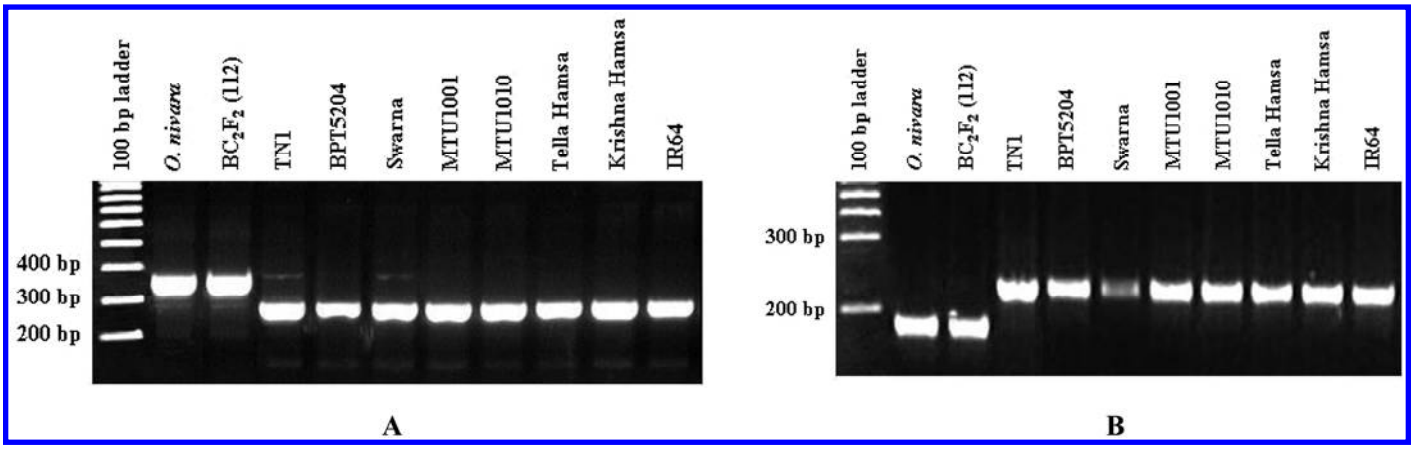

Fig. 3. Analysis of amplification pattern of the simple-sequence repeat (SSR) markers RMWR7.1 and RMWR7.6, which are closely linked to *Xa33*, in a set of eight high-priority bacterial blight (BB)-susceptible varieties ('TN1', 'BPT 5204', 'Swarna', 'MTU 1001', 'MTU 1010', 'Tella Hamsa', 'Krishna Hamsa', and 'IR 64') along with the donor parent International Rice Germplasm Collection (IRGC) 105710 and a BC_2F_2 plant (number 112) in the genetic background of 'Samba Mahsuri', which is homozygous for *Xa33*. Both **A,** RMWR7.1 and **B,** RMWR7.6 exhibited clear polymorphism between the donor genotypes for *Xa33* (i.e., IRGC 105710 and BC_2F_2 plant number 112) and the BB-susceptible elite rice varieties (TN1, BPT 5204, Swarna, MTU 1001, MTU 1010, Tella Hamsa, Krishna Hamsa, and IR 64) and, hence, are highly useful for marker-assisted selection.

Due to the problem of partial spikelet sterility and nonsurvival of many F_2 plants, we could not utilize the F_2 population derived from the cross IRGC 105710/TN1 for inheritance and gene mapping studies. Instead, the BC_1F_2 population derived from the cross IRGC 105710/TN1//TN1 was used for inheritance and mapping studies. Based on the segregation of BB resistance in the BC_1F_2 population, it was inferred that a single, dominant gene controlled BB resistance in the *O. nivara* (accession number IRGC 105710). No other BB resistance gene has been mapped on this chromosome except a recessive gene, *xa8*, which was coarsely mapped in the vicinity of the SSR marker RM214, which is located in the 12.7-Mb region on chromosome 7 (26). Therefore. the BB resistance gene derived from *O. nivara*, in the present study, was considered to be a new one, based not only on its genomic position on chromosome 7 but also on its differential resistance spectrum when compared with *xa8* as well as its nonallelic nature to *xa8* and other known resistance genes, as revealed from the allelic tests. Based on these observations, the gene can be considered as novel. The new gene has been designated as *Xa33* (22) and has been registered online at Oryzabase (http://shigen.lab.nig.ac.jp/rice/oryzabase_submission/gene_nomenclature/). Recently, this nomenclature has been approved by the Committee on Gene Symbolization, Nomenclature, and Linkage of Rice Genetics Cooperative (http://www.shigen.nig.ac.jp/rice/oryzabase_submission/gene_nomenclature/geneSymbol.do#X).

Fine mapping of the novel BB resistance gene was carried out using a set of SSR markers located on chromosome 7. Earlier studies in rice have also used SSR markers for mapping genes of interest (4,13,23). As in the case of previous reports (4,33,37), the strategy of BSA was successfully utilized in identifying the tentative location of the new BB resistance gene on chromosome 7. Because the genomic interval spanned by RM5711 and RM6728 had only a few SSR markers from the RM series of the rice SSR marker database (http://www.gramene.org), we designed a set of 10 new SSR markers based on the repeat motifs present in the region of interest, and these markers (RMWR7.1 to RMWR7.10) have not been reported earlier in the rice SSR database. Utilizing the RM series SSR markers and the newly designed SSR markers, we were able to identify at least four closely linked SSR markers (RMWR7.1, RMWR7.6, RMWR7.10, and RM21077) located in the 4-Mb genomic region on chromosome 7. Progeny testing at BC_1F_3 and BC_2F_3 generations ensured correct identification of homozygotes and heterozygotes among the BC_1F_2 and BC_2F_2 lines with no escapes, thus facilitating accurate genotyping with SSR markers in the process of mapping *Xa33*.

The utility of the closest flanking SSR markers, RMWR7.1 and RMWR7.6, was validated in an alternative BC_1F_2 mapping population derived from the cross IRGC 105710/Samba Mahsuri//Samba Mahsuri. It was observed that the map location and linkage distances of the SSR markers were similar to those observed in the BC_1F_2 mapping population derived from the cross IRGC 105710/TN1//TN1. Further, to utilize the novel gene *Xa33* for resistance to *X. oryzae* pv. *oryzae* effectively in marker-assisted breeding programs, we studied the amplification pattern of the flanking markers RMWR7.1 and RMWR7.6 in a set of eight varieties which were susceptible to BB and could serve as possible recipients for the new gene, *Xa33*. Both of the flanking SSR markers displayed unique polymorphism between resistant donors and susceptible rice varieties and, hence, can be considered useful for marker-assisted introgression of *Xa33* to a wide array of varieties susceptible to BB.

The marker RMWR7.1 was located at a genetic distance of 0.9 cM on one side and the marker RMWR7.6 at a distance of 1.2 cM on the other side flanking the BB resistance gene, *Xa33*. Anchoring the forward primer of RMWR7.1 and the reverse primer of RMWR7.6 on the physical map of the rice genome, the genomic region spanned by the two markers was observed to be ≈49 kb long. In an earlier study, while mapping *Xa30(t)*, the two markers Os04g53060 and Os04g53120 were observed to be at a genetic distance of 0.2 and 0.9 cM, respectively, from the gene and flanking and encompassing a region of 38.4 kb (4). Similarly, while mapping *Xa31(t)* on chromosome 4, the two closest restriction fragment length polymorphism markers, G235 and C600, were flanking the gene at a genetic distance of 0.2 cM (34). Using these genetic distances, (9,36), we have attempted in silico identification of putative candidates for the target genes. The genomic region of the Japonica rice genome (pseudomolecule 5, consisting of ≈49 kb) flanked by the closely linked SSR markers RMWR7.1 and RMWR7.6 was downloaded and analyzed for putative candidate genes by using the software FGENESH (http://www.softberry.com). In total, eight putatively expressed genes were identified in the region analyzed and, among these, a gene encoding serine-threonine kinase appears to be the most probable candidate for *Xa33.* Interestingly, the gene encoding serine-threonine kinase protein showed significant homology with the wheat rust resistance protein *Yr10* (29). Kinases are important signaling molecules in diverse biological systems. Zhou et al. (40) reported that the tomato *Pti1* gene conferring resistance against *Pseudomonas syringae* encodes a serine/threonine kinase and undergoes phosphorylation after interaction with the pathogen-encoded protein Pto and, as a result, induces the hypersensitive response. Two leucine-rich-repeat receptor-like kinase proteins, *Xa21* and *FLS2*, have a cytoplasmic serine/threonine kinase region (8,28). The kinase and Pto domains of *Xa21* and *FLS2* are involved in signal transduction and are essentially required for disease resistance. Further, the barley resistance protein *Rpg1* encoding resistance to stem rust disease also contains serine/threonine kinase domain (3). The functionality of the serine-threonine kinase gene identified in the present study as one of the possible putative candidates for *Xa33* needs to be investigated through genetic transformation studies.

A novel gene for resistance to *X. oryzae* pv. *oryzae*, *Xa33*, has identified from an accession of the wild rice *O. nivara*, tagged, and fine mapped on chromosome 7 using SSR markers, setting the stage for routine marker-assisted introgression of *Xa33* into elite BB-susceptible rice varieties. Further, a putatively expressed gene encoding a serine-threonine kinase which could be a po-

TABLE 3. List of putatively expressed genes in the genomic region flanked by simple-sequence repeat (SSR) markers RMWR7.1 and RMWR7.6 which are flanking the *Xa33* gene

Gene number	Gene start (bp)	Gene end (bp)	Gene size (bp)	Number of amino acids	Putative function of protein encoded by the gene and proteins showing homology to the gene
1	4,082,673	4,086,404	1,665	554	Serine threonine kinase super family
2	4,088,490	4,091,220	1,713	570	Putative transposon protein, mutator protein
3	4,100,292	4,102,188	843	280	Ribose-5-phosphate isomerase type A subfamily
4	4,102,967	4,108,223	2,340	779	Domain of unknown function 295
5	4,109,907	4,118,876	3,150	1,049	DNA repair/transcription protein Mms 19 like protein, no putative conserved domain have been detected
6	4,119,783	4,111,156	663	220	Base inhibitor (BI)-1-like family
7	4,121,693	4,124,083	735	244	BI-1-like superfamily
8	4,125,088	4,125,579	492	163	No putative conserved domains detected, hypothetical protein

tential candidate for *Xa33* has also been identified through in silico analysis, which can lead to map-based cloning of the gene.

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