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ORIGINAL PAPER

High-resolution genetic mapping of bacterial blight resistance gene *Xa10*

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Abstract Bacterial blight of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is the most devastating disease of rice (*Oryza sativa* L). Rice lines that carry resistance (*R*) gene *Xa10* confer race-specific resistance to *Xoo* strains harboring avirulence (*Avr*) gene *avrXa10*. Here we report on genetic study, disease evaluation and fine genetic mapping of the *Xa10* gene. The inheritance of *Xa10*-mediated resistance to PXO99^{A}(pHM1avrXa10) did not follow typical Mendelian inheritance for single dominant gene in F_2 population derived from IR24 × IRBB10. A locus might be present in IRBB10 that caused distorted segregation in F_2 population. To eliminate this locus, an F_3 population (F_3-65) was identified, which showed normal Mendelian segregation ratio of 3:1 for resistance and susceptibility. A new near-isogenic line (F_3-65-1743) of *Xa10* in IR24 genetic background was developed and designated as IRBB10A. IRBB10A retained similar resistance specificity as that of IRBB10 and provided complete resistance to PXO99^{A}(pHM1avrXa10) from seedling to adult stages. Linkage analysis using existing RFLP markers and F_2 mapping population mapped the *Xa10* locus to the proximal side of E1981S with genetic distance at 0.93 cM. With five new RFLP markers developed from the genomic sequence of Nipponbare, *Xa10* was finely mapped at genetic distance of 0.28 cM between proximal marker M491 and distal marker M419 and co-segregated with markers S723 and M604. The physical distance between M491 and M419 on Nipponbare genome is 74 kb. Seven genes have been annotated from this 74-kb region and six of them are possible *Xa10* candidates. The results of this study will be useful in *Xa10* cloning and marker-assisted breeding.

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Introduction

Bacterial blight of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most destructive bacterial diseases of rice and it is especially prevalent in irrigated and rainfed lowland rice growing areas throughout Asia (Mew 1987; Gnanamanickam et al. 1999). The yield losses caused by bacterial blight typically range from 20 to 30% but, in severely infested fields, the disease can cause as high as 50% yield reduction (Ou 1985).

The utilization of resistant varieties carrying *R* genes is one of the most effective methods to control this disease. The race-specific interaction between rice and *Xoo* is thought to follow the classic gene-for-gene concept (Flor 1971), in which the plant *R* gene product can recognize or interact with elicitor molecule, presumably encoded by an avirulence (*avr*) gene from the pathogen. In the absence of either the *avr* or the *R* gene or both, no recognition takes place and disease occurs (Flor 1971). Currently, about 30 *R* genes or loci against *Xoo* have been identified in cultivated and wild rice. Most of these genes provide complete and race-specific resistance to *Xoo* and have been used in rice breeding for bacterial blight resistance (Kinoshita 1995; Lin et al. 1996; Zhang et al. 1998; Khush and Angeles

1999; Gao et al. 2001; Chen et al. 2002; Yang et al. 2003; Gu et al. 2004). Genetic and physical mapping of these *R* genes not only permit marker-assisted breeding in rice, but also facilitate isolation and characterization of these genes at the molecular level. So far, five dominant *R* genes, *Xa21* (Song et al. 1995), *Xa1* (Yoshimura et al. 1998), *Xa26* (Sun et al. 2004), *Xa27* (Gu et al. 2005) and *Xa3* (Xiang et al. 2006) and two recessive *R* genes, *xa5* (Iyer and McCouch 2004) and *xa13* (Chu et al. 2006), have been isolated by map-based cloning.

The *R* gene *Xa10* was originally identified from rice cultivar Cas 209 (Mew et al. 1982; Yoshimura et al. 1983). *Xa10* confers race-specific resistance to only a few Philippine races of bacterial blight pathogens such as PXO86 (R2), PXO112 (R5) and PXO145 (R7) (Yoshimura et al. 1995). *Xa10* was later introgressed into susceptible rice variety IR24 and a near-isogenic line of *Xa10*, IRBB10, was developed for determining the resistance specificity of the *R* gene (Ogawa et al. 1988). The cognate *avrXa10* gene isolated from *Xoo* strain PXO86 encodes a member of AvrBs3/PthA family of type-III effectors (Hopkins et al. 1992). The cloning of *avrXa10* greatly enhanced the understanding on gene-for-gene interactions and facilitated tagging of this resistance locus.

The *Xa10* locus was initially mapped to the long arm of chromosome 11 (11L) in the region between proximal RAPD marker $O07_{2000}$ (5.3 cM) and distal RFLP marker CDO365 (16.2 cM) (Yoshimura et al. 1995). The *R* locus was later integrated to the region between RFLP markers RG103 (~83 cM) and RG1109 (~91.4 cM) on rice genetic map of double haploid lines (IR64 and Azucena), however, no experiment was carried out to verify the map position (Ramalingam et al. 2003). Chromosome 11L of rice is rich in *R* genes for disease resistance to bacterial blight. In addition to *Xa10*, *Xa3* (Yoshimura et al. 1992a, b, 1995), *Xa4* (Yoshimura et al. 1992a, b, 1995), *Xa21* (Ronald et al. 1992), *Xa22(t)* (Lin et al. 1996), *Xa23* (Zhang et al. 1998) and *Xa26* (Yang et al. 2003) were also mapped to this chromosome. Among them, *Xa21, Xa26* and *Xa3* have been isolated and all of them encode leucine-rich repeat (LRR) receptor kinase-like proteins (Song et al. 1995; Sun et al. 2004; Xiang et al. 2006). *Xa3* is allelic to *Xa26* and they share 92% sequence identity. *Xa3/Xa26*, *Xa4* and *Xa22(t)* were mapped to the distal end of chromosome 11L. *Xa26* co-segregated with R1506 (116.2 cM) while *Xa4* was tightly linked with *Xa3* (Sun et al. 2004; Yoshimura et al. 1995). *Xa21* and *Xa23* reside at the middle region of chromosome 11L. *Xa21* co-segregated with RG103 (83.0 cM) (Ronald et al. 1992) while *Xa23* was mapped to a region between markers RG1109 (91.4–97.3 cM) and G1465 (99.2 cM) (Zhang et al. 1998). Based on the above information, we speculate that the *Xa10* locus is flanked by *Xa21* and *Xa23*. Both *Xa21* and *Xa23* conferred broad-spectrum resistance to multiple *Xoo* strains, including six races of the Philippine strains (Khush et al. 1989; Zhang et al. 2001).

In this study, we performed fine genetic mapping of the *Xa10* gene with a large mapping population using existing genetic markers as well as markers developed based on the published genomic sequence of Nipponbare. The resistance specificity of *Xa10* was also evaluated for different *Xoo* strains with a newly developed near-isogenic line. The molecular markers identified in this study will facilitate isolation of the *Xa10* gene by positional cloning and marker-assisted selection of the *R* gene in rice breeding.

Materials and methods

Plant materials and mapping populations

IRBB10 is a near-isogenic line of *Xa10* in IR24 genetic background (Ogawa et al. 1988). Using IRBB10 as male donor and susceptible line IR24 as recipient of *Xa10*, crosses were made for generation of two-related *Xa10* mapping populations. One population consisted of 1,027 F_2 individuals and another population contained 247 F_3 plants developed from heterozygous plant F_2-65 (Table 1). Rice plants, including those inoculated with *Xoo* strains, were grown in greenhouse at a temperature of 30°C for 12.5 h (light) and 26°C for 11.5 h (dark).

Table 1 Populations generated for fine genetic mapping of the *Xa10* locus

Population[a]	Plants		χ^2	*P*
	Resistant	Susceptible		
F_2	890	137	74.5	<0.001
F_3-4	464	11	130.4	<0.001
F_3-17	183	5	50.1	<0.001
F_3-19	157	3	45.6	<0.001
F_3-23	597	11	174.4	<0.001
F_3-35	95	1	29.4	<0.001
F_3-44	121	9	22.7	<0.001
F_3-65	189	58	0.304	0.5–0.6
F_4-1510	32	11	0.085	$0.75 < P < 0.8$
F_4-1531	90	31	0.025	$0.8 < P < 0.9$

[a] The F_3 populations were derived from heterozygous F_2 plants. The F_4 populations F_4-1510 and F_4-1531 were derived from heterozygous F_3 plants in F_3-65

The populations F_3-4, F_3-17, F_3-19, F_3-23, F_3-35, F_3-44, F_4-1510 and F_4-1531 were not included in linkage for genetic mapping of the *Xa10* locus

Bacterial blight inoculation

All *Xoo* strains were grown at 28°C on PSA (Peptone sucrose agar) plates except that 100 μg/ml spectinomycin was added to the medium for PXO99^{A}(pHM1avrXa10). The bacterial inoculum was prepared by suspending bacterial culture in sterile, distilled water at an optical density of 0.5 (OD_{600}). Bacterial blight inoculation was carried out according to leaf-clipping method (Kauffman et al. 1973). Disease scoring was measured as described previously (Gu et al. 2004).

Probes of RFLP markers

RFLP marker probes were amplified from rice variety Nipponbare by polymerase chain reaction (PCR). PCR amplification was carried out in a reaction of 20 μl containing 0.2 μM of each forward and reverse primers, 2.5 mM $MgCl_2$, 0.2 mM dNTP, 1× PCR buffer (50 mM KCl, 10 mM Tris, pH 8.3), 10–20 ng template DNA and 1 unit of *Taq* polymerase (Qiagen, Germany) in a PTC-100 thermal cycler (MJ Research, USA). PCR reaction was performed as follows: 94°C for 120 s; followed by 35 cycles at 94°C for 40 s, 55–60°C for 50 s, 72°C for 90 s and finally extended for 5 min at 72°C. The amplified markers were cloned into pGEM T-easy vector (Promega, USA) and verified by DNA sequencing. The DNA primers that were used to amplify the molecular markers are listed in Table 2.

Southern blot analysis

Rice genomic DNA was isolated from leaf tissues according to the procedures described previously (Dellaporta et al. 1983). About 2 μg of DNA was digested with the appropriate restriction enzymes, separated on 0.8% agarose gel and blotted to Hybond^{TM}-N + nylon membrane (Amersham Biosciences, GE healthcare, USA). DNA hybridization was conducted according to standard procedures (Sambrook et al. 1989). DNA probes were labeled with [^{32}P]-dCTP using Rediprime II random prime labeling system (Amersham Biosciences).

Results

Inheritance of *Xa10* and generation of mapping populations

Our preliminary data indicated that the inheritance of *Xa10* showed distortion in the segregation of resistance and susceptibility in different F_2 populations. The segregation ratio was 7:1–9:1 when original incompatible *Xoo* strains PXO86 (R2) was chosen to phenotype for *Xa10*, which did not follow Mendelian segregation ratio of 3:1 for inheritance of single dominant locus (data not shown). To investigate whether there are two linked *R* genes that might recognize different *avr* gene products from PXO86 or the same AvrXa10 effector, we chose PXO99^{A} (pHM1avrXa10) to phenotype for *Xa10*. IRBB10 was susceptible to PXO99^{A} but showed complete resistance to PXO99^{A} (pHM1avrXa10) that carried the *avrXa10* gene in a cosmid plasmid (Table 3; Yang et al., 2000). The recurrent parental line IR24 was susceptible to both *Xoo* strains (Table 3). These results indicate that rice plants with *Xa10* showed race-specific resistance to PXO99^{A} that harbors *avrXa10*. PXO99^{A} (pHM1avrXa10) was then used in phenotyping *Xa10* during genetic analysis and mapping population development. The F_1 plants derived from the crosses between IR24 and IRBB10 conferred complete resistance to PXO99^{A}(pHM1avrXa10) (data not shown). A large F_2 population was generated for genetic analysis and mapping of the *Xa10* locus. Phenotypic evaluation showed that the F_2 population consisted of 890 resistant and 137 susceptible plants. This segregation still did not follow Mendelian segregation ratio of 3:1 for inheritance of single dominant locus ($\chi^2 = 74.5$, $P < 0.001$) (Table 1). This distortion in the *Xa10* inheritance might be due to a locus that is linked to *Xa10* and affected the fertility or survival of susceptible F_2 zygotes (Genotype: *xa10/xa10*). The presence of this

Table 2 Molecular markers developed for fine genetic mapping of *Xa10*

Marker	PCR primer (5′-3′)[a]	Size (bp)	Restriction enzyme[b]
M582	F: CTTGATTTCTAACTCTTCTGCTC R: TTTGTCTTTCTCTCTATGCAGG	582	*Hin*dIII or *Dra*I
M491	F: AGTAATGGATGCAGTGTGGGGC R: TTGTTTGCTCACTCCACCCTTC	491	*Sty*I
M604	F: CAACGCCTATCTTCTGCATTTC R: GTGACCCTAGTTTCTGGTTATG	604	*Xba*I
M419	F: CATCAGCAACCCCGTGAAAACG R: GATGGCAATGTACCGCGAATAC	419	*Pvu*II
M593	F: TCAGGGAGATGTTCTGGCCTTC R: CTTATTCGAGGATAGTCGTCAC	593	*Ava*II

[a] Primers used to amplify molecular markers from Nipponbare. F, forward primer; R, reverse primer

[b] Restriction endonuclease that revealed polymorphism between IR24 and IRBB10 in RFLP analysis

Table 3 Disease evaluation of IRBB10 and F_3-65-1743 (IRBB10A) to *Xoo* strains

Xoo strain	Origin	Lesion length (cm) and resistance score[a]		
		IRBB10	IRBB10A	IR24
PXO99^A	Philippines	23.7 ± 2.5 (S)	22.2 ± 3.8 (S)	23.6 ± 3.3 (S)
PXO99^A(pHM1avrXa10)[b]	Yang et al. (2000)	0.2 ± 0.2 (R)	0.2 ± 0.1 (R)	28.2 ± 3.8 (S)
PXO86 (R2)	Philippines	0.1 ± 0.0 (R)	0.4 ± 0.4 (R)	21.4 ± 3.2 (S)
PXO112 (R5)	Philippines	0.1 ± 0.0 (R)	0.1 ± 0.0 (R)	12.8 ± 3.0 (S)
Aust-2031	Australia	5.3 ± 1.2 (MR)	3.6 ± 1.5 (MR)	5.2 ± 1.1 (MR)
Aust-R3	Australia	7.5 ± 1.6 (MS)	6.1 ± 2.2 (MS)	5.5 ± 1.3 (MR)
R-7	Thailand	6.5 ± 3.1 (MS)	7.3 ± 2.1 (MS)	10.1 ± 6.7 (S)
PXO79 (R3)	Philippines	17.9 ± 3.3 (S)	22.9 ± 3.4 (S)	23.3 ± 4.3 (S)
PXO71 (R4)	Philippines	23.4 ± 5.4 (S)	23.0 ± 4.0 (S)	23.4 ± 3.3 (S)
PXO113 (R4)	Philippines	19.7 ± 3.8 (S)	17.6 ± 3.2 (S)	18.0 ± 2.5 (S)

[a] The lesion length and the standard deviation of the mean were the average of 16 infected leaves. For score: *R* resistant, 0 cm < LL < 3.0 cm; MR, moderately resistant, 3.0 cm < LL < 6.0 cm; MS, moderately susceptible, 6.0 cm < LL < 9.0 cm; S, susceptible, lesion length > 9.0 cm

[b] The strain PXO99^A(pHM1avrXa10) was designated as PXO99(pZWavrXa10) in Yang et al. (2000)

putative locus is assumed on the fact that more of the heterozygous F_2 plants (Genotype: *Xa10/xa10*) but only a few of the homozygous plants (Genotype: *Xa10/Xa10* or *xa10/xa10*) showed partial fertility (Table 4).

The putative locus was able to be separated from *Xa10* in subsequent generations. Seven F_2 plants that were heterozygous at the *Xa10* locus were randomly selected to generate F_3 segregation populations. One of the F_3 populations (F_3-65) showed typical Mendelian segregation ratio of 3:1 for inheritance of single dominant *R* gene against PXO99^A (pHM1avrXa10) (R:S = 189:58, $\chi^2 = 0.304$, $0.5 < P < 0.6$) (Table 1). The remaining six F_3 segregation populations still showed distortion for segregation of resistance and susceptibility (Table 1). The inheritance of the resistance locus as single dominant *R* gene to PXO99^A (pHM1avrXa10) in F_3-65 was further verified from the phenotypic evaluation of two F_4 segregation populations (F_4-1510, R:S = 32:11, $\chi^2 = 0.085$, $0.75 < P < 0.8$; F_4-1531, R:S = 90:31, $\chi^2 = 0.025$, $0.8 < P < 0.9$) (Table 1).

To further investigate whether the resistance locus in the F_3-65 population still retains *Xa10* resistance specificity, a homozygous plant (F_3-65-1743) from the F_3-65 population was inoculated with various *Xoo* strains. Disease evaluation showed that line F_3-65-1743 retained similar resistance specificity as that of IRBB10 (Table 3). Line F_3-65-1743 can be used as a new near-isogenic line of *Xa10* in IR24 genetic background, which was designated as IRBB10A. The influence of plant development on *Xa10*-mediated resistance to bacterial blight was investigated by inoculating lines IRBB10A and IR24 with PXO99^A(pHM1avrXa10) at different developmental stages. IRBB10A conferred complete resistance to PXO99^A(pHM1avrXa10) from seedling to adult stages (Fig. 1).

Table 4 Summary of fertility of the F_2 plants in the mapping population

Genotype	Complete fertile plants	Partial fertile plants
Xa10/Xa10	365	7
Xa10/xa10	318	200
xa10/xa10	131	6

Fertility was scored according to the following standard: complete fertile, above 90% of spikelets on each panicle were set with seeds; partile fertile, less than 90% of spikelets on each panicle were set with seeds

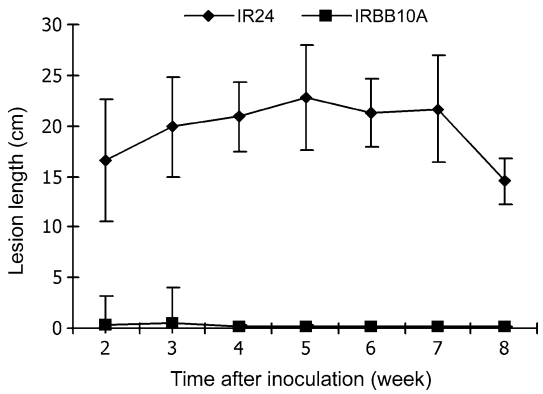

Fig. 1 Resistance of *Xa10* to *Xoo* strain PXO99^A(pHM1avrXa10) at different developmental stages. New developed near-isogenic line IRBB10A of *Xa10* in IR24 genetic background and recurrent susceptible parental line IR24 were inoculated with PXO99^A(pHM1avrXa10) at different developmental stages from 2 to 8 weeks. Bacterial inoculation was carried out as described in the text. Only fully expanded leaves of the main culm (from seedling to active tillering stage) or each tiller (after active tillering stage) were selected for inoculation. The lesion lengths of bacterial blight are the average values of 8–24 inoculated leaves with standard deviations

Table 5 Survey of polymorphism between IRBB10 and IR24 using molecular markers on long arm of rice chromosome 11

Marker	Genetic location (cM)	Polymorphism	Restriction enzymes
C3	56.2	Yes	*Acc*I, *BstE*II
RG103	83.0	ND	ND
E1126S	84.3	No	–
RZ537	84.6	No	–
C189	85.7	No	–
S723	85.7	Yes	*Xba*I, *Stu*I
E1981S	88.4	Yes	*Hin*dIII, *Sty*I
S10928	89.0	Yes	*Stu*I, *Sph*I
C50	89.0–90.6	Yes	*Hin*dIII, *Nco*I
RG1109	91.4	ND	ND
R1506	116.2	Yes	*Sty*I

ND not detected

Genetic mapping of the *Xa10* locus with existing RFLP markers

Previously, the *Xa10* locus was roughly integrated to a large region between RFLP markers RG103 (~83 cM) and RG1109 (~91.4 cM) on the long arm of rice chromosome 11 (Ramalingam et al. 2003). To detect the introgressed region in IRBB10, nine existing RFLP markers on the long arm of chromosome 11 were surveyed for polymorphism between IRBB10 and IR24 with 30 restriction enzymes. Out of seven markers flanked by RG103 and RG1109, four markers showed polymorphism covering a genetic region from 85.7 to 90.6 cM (Table 5). Markers E1126S (84.3 cM), RZ537 (84.6 cM) and C189 (85.7 cM) did not show any polymorphism (Table 5). The other two markers, C3 (56.2 cM) to the proximal side of RG103 and R1506 (116.2 cM) to the distal side of RG1109, showed polymorphism (Table 5). The polymorphism between IRBB10 and IR24 detected by these RFLP markers indicated that there were more than one introgressed regions from Cas 209 on chromosome 11L of IRBB10. The *Xa10* locus may be located in the introgressed region between C189 to at least C50 at the distal end of chromosome 11L. To verify this, we carried out linkage analysis between marker E1981S and the *Xa10* locus with 1,027 individuals in F_2 population (Table 1). Nineteen recombinants were identified by E1981S and all these recombinants were complemented by marker S723 (Fig. 2). Thus, the *Xa10* locus was mapped to the proximal side of marker E1981S with genetic distance at 0.93 cM and co-segregated with marker S723 (Fig. 2).

Saturation of the *Xa10* locus with new developed RFLP markers

To further saturate the *Xa10* locus with additional molecular markers, five new RFLP markers were developed based on the available genomic sequence of Nipponbare (Table 2). These markers were used as probes in RFLP analysis to survey polymorphism between IRBB10 and IR24 using 30 restriction enzymes (Table 2). Linkage analysis was carried out using these markers and recombinants were identified from F_2 and F_3 mapping populations. M582 and M491 were the two proximal markers with six and one recombinant identified, respectively. M491 was the closest proximal marker to the *Xa10* locus with genetic distance of 0.04 cM (Fig. 2). M419 and M593 were identified as two distal markers. Whereas M419 complemented thirteen of the nineteen E1981S recombinants, the marker M593 complemented only nine. Therefore, M419 was the nearest distal marker to the *Xa10* locus with genetic distance at 0.24 cM. Another marker M604 co-segregated with the *Xa10* locus.

In conclusion, the *Xa10* locus was genetically mapped to the region between proximal marker M491 (0.04 cM) and distal marker M419 (0.24 cM). The *Xa10* locus on the genome of Nipponbare is covered by RGP PAC clone P0480H08 (AC104847) and overlapped with CUGI BAC clone OSJNBa0029K08 (AC136148) (http://www.rgp.dna.affrc.go.jp/cgi-bin/statusdb/irgspMini.pl?chr=11) (Fig. 2). The physical distance between M491 and M419 on the genome of Nipponbare is 74 kb (Fig. 2).

Xa10 candidate genes

Within the 74-kb region, seven genes including one encoding 5S ribosomal RNA have been annotated (http://www.ncbi.nlm.nih.gov/entrez/viewer.fcgi?db=nuccore&id=58531198). The remaining six genes encode a protein kinase (BAF 28530), a conserved hypothetical protein (BAF28531), a DUF231 domain containing protein with unknown function (BAF28532), an ADP-ribosylation factor (BAF28533), a TPR-like domain containing protein (BAF28534) and a hypothetical protein (BAF28535), respectively. Three of them (BAF28530, BAF28531 and BAF28533) were supported by evidence of transcription. All these six expressed or predicted proteins are possible *Xa10* candidates (Fig. 2), which are discussed in the discussion section. Gene density within this region is about one gene every 10.9 kb, consistent with published predictions of one gene every 9.9 kb (International Rice Genome Sequencing Project 2005). However, it is worthy to mention that no gene was annotated in the 29-kb region between the two genes that encode BAF28531 and BAF28532 even though most of the DNA sequence in the 29-kb region possesses single copy in Nipponbare genome.

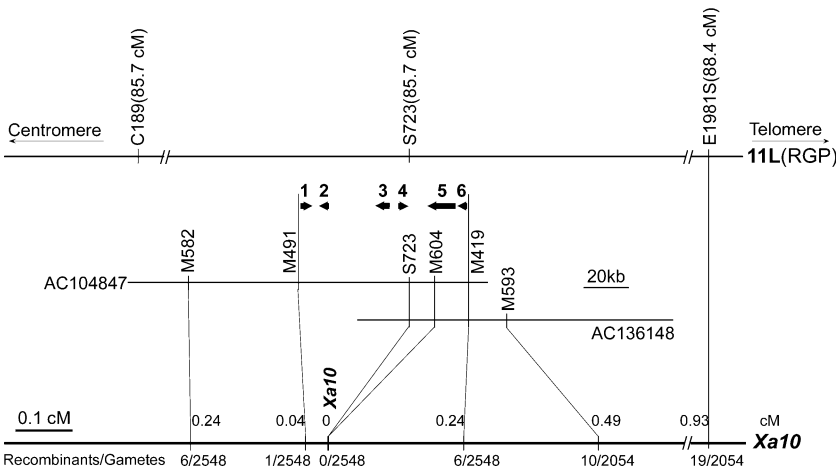

Fig. 2 High-resolution genetic map of the *Xa10* locus. Genetic map of *Xa10* based on linkage analysis is shown in the diagram below and recombinants/gametes are indicated. Genetic and physical maps of Rice Genome Research Program (*RGP*) at the syntenic position of the *Xa10* locus are also shown. Genetic distance is given in centimorgan (*cM*). The accession numbers of cv. Nipponbare sequences are indicated for each BAC/PAC clone. The positions of the respective markers on genetic and physical maps are denoted with lines. *Bars* for genetic and physical distances are indicated. Six putative *Xa10* candidate genes, which were annotated by International Rice Genome Sequencing Project (*IRGSP*), are shown above the physical map between makers M491 and M419 with arrows indicating the orientation of transcription. Proteins encoded by these candidate genes are as follows: *1* protein kinase (BAF28530), *2* conserved hypothetical protein (BAF28531); *3* DUF231 domain containing protein with unknown function (BAF28532), *4* ADP-ribosylation factor (BAF28533), *5* TPR-like domain containing protein (BAF28534), *6* hypothetical protein (BAF28535). The gene encoding 5S ribosomal RNA, which was annotated between *Xa10* candidate gene 5 and 6, is not shown

Discussion

IRBB10 was developed as a near-isogenic line of the *Xa10* gene in IR24 genetic background (Ogawa et al. 1988). However, there was extensive polymorphism on chromosome 11 L between IRBB10 and IR24. In addition, we found that there is great distortion in the *Xa10* inheritance. The distortion was not due to the presence of two linked *R* genes that recognize the same AvrXa10 from PXO99^{A} (pHM1avrXa10) as the two closely linked RFLP markers, S723 and M604, always co-segregated with resistant plants (*Xa10* plants) in linkage analysis. A putative locus, which might come from Cas 209 and links with *Xa10* in IRBB10, affected the fertility of heterozygous *Xa10* plants, resulted in fewer susceptible progeny, and, therefore, caused the distortion in the *Xa10* inheritance. Elimination of this locus as well as the other unrelated introgressed regions did not change *Xa10* resistance specificity, which led to the development of better near-isogenic line of the *Xa10* in IR24 genetic background. The high-resolution genetic map at the *Xa10* locus and closely linked markers identified in this study will facilitate the isolation of the *Xa10* gene by positional cloning as well as marker-assisted selection of the *R* gene in breeding program.

The high-resolution mapping allowed us to narrow down *Xa10* to the region between RFLP markers M491 and M419. The corresponding physical distance between these two markers on Nipponbare genome is 74 Kb. This region has seven genes and six of them encode possible *Xa10* candidates with protein identity numbers from BAF28530 to BAF28535 in Genbank.

BAF28530 is a putative serine/threonine kinase (STK). So far, only Pto, a *R* protein from tomato, was identified to be serine/threonine-protein kinase (Martin et al. 1993). Two leucine-rich-repeat (LRR) receptor-like kinase proteins, XA21 and FLS2, have a cytoplasmic serine/threonine kinase region (Gomez-Gomez and Boller 2000; Song et al. 1995). Both Pto and the kinase domains of XA21 and FLS2 are involved in signal transduction and required for resistance. In addition, the barley *R* protein Rpg1 for resistance to stem rust also contains serine/threonine kinase domains (Brueggeman et al, 2006). However, BAF28530 shows very low similarity to these kinases.

BAF28531 shows medium level of similarity to RNA polymerase II transcription elongation factor DSIF/SUPT5H/SPT5 (ISS) of the smallest free-living eukaryote *Ostreococcus tauri* (Derelle et al. 2006). Although none of the transcription elongation factors of host plants are known to be involved in disease response, it has been shown that bacterial elongation factor Tu (EF-Tu), which is perceived by receptor kinase EFR (Zipfel et al. 2006), elicits innate immunity in *Arabidopsis* (Kunze et al. 2004). In another example, mutations in ABO1/ELO2, a subunit of holo-elongator, increase abscisic acid sensitivity and drought tolerance in *Arabidopsis* (Chen et al. 2006).

The amino acid sequence of BAF28532 shows high identity to several uncharacterized leaf senescence protein-like proteins of rice. It contains two DUF231 domains

(DUF, domain of unknown function). Proteins with DUF domains belong to a large protein family in *Arabidopsis thaliana* as well as in rice. *Arabidopsis* ESK1 has a conserved DUF231 domain and its mutation showed freezing tolerance (Xin and Browse 1998; Xin et al. 2007).

BAF28533 is an ADP-ribosylation factor (ARF)-like protein. The small G proteins of the ARF family are key regulators of membrane dynamics and vesicle trafficking (Nie et al. 2003; Memon 2004). Accelerated vesicle traffic is associated with a polarized cell wall-associated defense in plants (Bestwick et al. 1995; Collins et al. 2003). Components in plant vesicle trafficking pathway may also be targeted by virulence factors of pathogens. Recently, HopM1, a conserved *Pseudomonas syringae* virulence protein, was found to target an ARF-associated protein, AtMIN7, in *Arabidopsis thaliana* and mediate the destruction of AtMIN7 via the host proteasome (Nomura et al. 2006). ARF was also found to be up regulated in resistant cultivar Remo of domestic apple (*Malus domestica*) (Degenhardt et al. 2005). Over-expression of *NtARF* or rice ADP-ribosylation factor 1 in tobacco plants induced pathogenesis-related genes and exhibited hypersensitive response phenotype and enhanced resistance to pathogen attack (Lee et al. 2003; Lee and Sano 2007).

BAF28534 contains 12 tandem pentatricopeptide repeats (PPRs). PPRs are degenerate motifs, each with 35-amino-acid sequences, and are present in tandem arrays of 2–27 repeats per protein (Saha et al. 2007). Functional studies on different PPR proteins have revealed their role in organellar RNA processing, fertility restoration in CMS plants, embryogenesis, and plant development (Saha et al. 2007). PPR proteins hold common features with most abundant type of plant *R* proteins, nucleotide binding site and leucine-rich repeats (NBS-LRR) type *R* proteins (Geddy and Brown 2007). However, so far, no PPR protein was reported to be involved in plant defense.

Finally, BAF28535 is a hypothetical protein with no functional domain predicted based on its amino acid sequences.

Recently, the AvrXa10-like type-III effectors were also described as transcription activator-like (TAL) effectors (Yang et al. 2006; Sugio et al. 2007). TAL effectors each possess central repetitive regions that vary in repeat number and specific repeat sequence as well as three highly conserved C-terminal nuclear localization signals (NLS), and C-terminal acidic transcription activator-like domain (AD). TAL effectors are associated with phenotypes that contribute to strain virulence, disease symptoms, and host recognition and resistance responses, the latter also known as avirulence activity. So far, the induction of four host genes has been characterized to be specifically regulated by the TAL effectors from *Xoo* strain PXO99^{A} (Gu et al. 2005; Sugio et al. 2007; Yang et al. 2006). For example, expression of the dominant rice *R* gene *Xa27* is specifically induced by AvrXa27 and resulted in a hypersensitive resistance reaction (Gu et al. 2005). However, the *Xa27* protein (XA27) is unrelated to any previously characterized *R*-gene products. The structural analysis of XA27 provides little or no clues as to the mode-of-action of the protein. In another example, the specific induction of rice gene *Os8N3* by PthXo1 is required for full strain virulence and increased host disease susceptibility, respectively (Yang et al. 2006). The recessive alleles of *Os8N3*, collectively known as *xa13*, interferes with *Os8N3* induction during infection by PXO99^{A} and results in resistance to strains that rely on PthXo1 for virulence (Chu et al. 2006; Yang et al. 2006). Interestingly, the *Os8N3/XA13* protein, which is a plasma membrane associated protein of MtN3 family, is also required for pollen development. Recently, the rice *OsTFX1*, a bZIP transcription factor gene, and *OsTFIIAγ1*, a gene encoding the small subunit of the transcription factor IIA, were identified to be specifically induced by TAL effectors PthXo6 and PthXo7, respectively, for host susceptibility to disease (Sugio et al. 2007). In all these cases, it is their promoters that determine resistance or susceptibility specificity and may be recognized by the TAL effectors, while the gene products show great diversity. Previous studies indicate that AvrXa10 has TAL effector function (Zhu et al. 1998, 1999). Based on the protein features of *Xa10* candidates and AvrXa10 function, the *Xa10* gene may encode a new kind of *R* protein for resistance to bacterial blight, whose expression may be regulated by AvrXa10.

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