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ORIGINAL PAPER

High-resolution genetic mapping of rice bacterial blight resistance gene *Xa23*

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Abstract Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae (Xoo)* is the most devastating bacterial disease of rice (*Oryza sativa* L.), a staple food crop that feeds half of the world's population. In management of this disease, the most economical and effective approach is cultivating resistant varieties. Due to rapid change of pathogenicity in the pathogen, it is necessary to identify and characterize more host resistance genes for breeding new resistant varieties. We have previously identified the BB resistance (*R*) gene *Xa23* that confers the broadest resistance to *Xoo* strains isolated from different rice-growing regions and preliminarily mapped the gene within a 1.7 cm region on the long arm of rice chromosome 11. Here, we report fine genetic mapping and in silico analysis of putative candidate genes of *Xa23*. Based on F_2 mapping populations derived from crosses between *Xa23*-containing rice line CBB23 and susceptible varieties JG30 or IR24, six new STS markers Lj36, Lj46, Lj138, Lj74, A83B4, and Lj13 were developed. Linkage analysis revealed that the new markers were co-segregated with or closely linked to the *Xa23* locus. Consequently, the *Xa23* gene was mapped within a 0.4 cm region between markers Lj138 and A83B4, in which the co-segregating marker Lj74 was identified. The corresponding physical distance between Lj138 and A83B4 on Nipponbare genome is 49.8 kb. Six *Xa23* candidate genes have been annotated, including four candidate genes encoding hypothetical proteins and the other two encoding a putative ADP-ribosylation factor protein and a putative PPR protein. These results will facilitate marker-assisted selection of *Xa23* in rice breeding and molecular cloning of this valuable *R* gene.

Keywords *Xanthomonas oryzae* pv. *oryzae* · *Xa23* · Fine mapping · Rice

Introduction

Bacterial blight (BB), caused by *Xanthomonas oryzae* pv *oryzae (Xoo)*, is one of the most serious and destructive diseases of rice (*Oryza sativa* L.). This disease has been attracting many researchers, because it is not only important for rice production worldwide, but also a model system to investigate plant–bacteria interactions (Niño-Liu et al. 2006). As to the management of BB in rice production, adoption of host resistance is practically proven the most economical, effective, and eco-friendly approach (Suh et al. 2013). Therefore, scientists have been putting great efforts to identify BB resistance (*R*) genes from both cultivated rice varieties and wild rice species (Brar and Khush 1997; Kumar et al. 2012). We have previously identified a broad-spectrum BB resistance gene *Xa23* from the wild rice *Oryza rufipogon* (Zhang et al. 1998). Since the *Xa23* locus confers complete dominant and high resistance to virtually all *Xoo* races tested at all growth stages of rice (Zhang et al. 2002; Wang et al. 2013), it has been widely adopted in rice

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breeding programs (Zhou et al. 2009; Huang et al. 2012). In the present study, we conducted fine genetic mapping of *Xa23* to facilitate marker-assisted selection and molecular cloning of this valuable *R* gene.

Thus far, about 38 BB resistance genes (designated in a series from *Xa1* to *Xa38*) have been identified in rice (Lin et al. 1996; Khush and Angeles 1999; Chen et al. 2002, 2011; Blair et al. 2003; Lee et al. 2003a; Gu et al. 2004; Tan et al. 2004; Cheema et al. 2008; Korinsak et al. 2009; Wang et al. 2009; Zheng et al. 2009; Guo et al. 2010; Miao et al. 2010; Bhasin et al. 2012). Among the identified BB resistance genes, 11 (*xa5, xa8, xa13, xa15, xa19, xa20, xa24, xa26b, xa28, xa32, and xa34*) are recessive in inheritance (Chen et al. 2011) and the others are dominant in nature. Most of the identified BB resistance genes have been mapped to rice chromosomes, and eight of them have been molecularly cloned, including dominant genes *Xa1* (Yoshimura et al. 1998), *Xa3* or *Xa26* (Sun et al. 2004; Xiang et al. 2006), *Xa10* (Tian et al. 2014), *Xa21* (Song et al. 1995), *Xa27* (Gu et al. 2005) and recessive genes *xa5* (Iyer and McCouch 2004; Jiang et al. 2006), *xa13* (Chu et al. 2006) and *xa25* (Liu et al. 2011).

Although many BB resistance genes have been identified, most of them hold limited application value due to weak resistance, narrow resistance spectrum or recessive inheritance nature. Consequently, only a few of them, such as *Xa3*, *Xa4*, *Xa7,* and *Xa21*, have been practically used in rice production. In this regard, more effective BB resistance genes should be identified and characterized.

In our previous investigations, the *Xa23* locus has been transferred into a susceptible indica rice variety JG30, resulting in near-isogenic line CBB23 (Zhang et al. 2002). We have previously mapped the *Xa23* locus within a 1.7 cm region on the long arm of rice chromosome 11, between molecular markers CP02662 (Wang et al. 2005) and 69B (Wang et al. 2006). We here report genetic fine mapping and in silico analysis of putative candidate genes of *Xa23*.

Materials and methods

Plant materials

JG30 is an indica rice variety highly susceptible to all *Xoo* strains tested. CBB23 is a near-isogenic line of *Xa23* in genetic background of JG30 (Zhang et al. 2002). IR24 is a *Xoo* strain PXO99 susceptible indica rice variety from International Rice Research Institute. CBB23 was crossed as male to JG30 and IR24, respectively. The F_1 plants were self-pollinated to generate F_2 populations. Rice materials used for *Xa23* mapping were listed in Table 1. Additional 36 rice varieties/lines used for the polymorphism assays at *Xa23* locus were indicated in Fig. 3. All rice plants were grown in field or greenhouse at 28–35 °C in day light.

Table 1 Rice plants used for *Xa23* fine mapping and their reactions to *Xoo* strain PXO99

Rice material	R	S	Total	$\chi^2_{(3:1)}$	*P* value
JG30	0	30	30		
CBB23	30	0	30		
IR24	0	30	30		
(JG30×CBB23) F_2 population	1,930	632	2,562	0.13	0.72
(IR24×CBB23) F_2 population	810	266	1,076	0.03	0.86

R resistant, *S* susceptible

Bacterial inoculation and plant resistance assessment

The Philippine race 6 (PXO99) of *Xoo* was used to evaluate disease phenotypes of rice plants. *Xoo* cells were cultured in PPS medium [ferv-filtering juice of 300 g potato, 5 g peptone, 15 g sucrose, 2 g $Na_2HPO_4{\cdot}12H_2O$, and 0.5 g $Ca(NO_3)_2{\cdot}4H_2O$] at 28 °C for 48 h. Bacterial suspensions (OD_{600} = 1.0) with sterile distilled water were inoculated by the leaf-clipping method (Kauffman et al. 1973) in leaves of rice plants at booting stage. For each plant, 3–5 fully expanded leaves were inoculated. Disease symptom was scored 2 weeks post inoculation. The disease symptom was scored by lesion area ratio against the whole leaf through visual assessment (Wang et al. 2005). Plants with lesion areas equal to or less than 15 % were classified as resistant (R) and those with lesion areas larger than 15 % were classified as susceptible (S) plants.

Extraction of genomic DNA and PCR procedure

Genomic DNA of 39 rice varieties/lines and individual plants of the F_2 populations were isolated from leaves following the description by McCouch et al. (1988). PCR amplification was carried out with 20 μL reaction volume containing 2 μL of 10× PCR buffer, 1.2 μL of dNTPs (10 mmol L^{-1}), 0.3 μL of each primer (10 μmol L^{-1}), 50 ng of DNA template, 1.0 U of Taq DNA polymerase. The reactions were heated to 94 °C for 3 min followed by 35 cycles of amplification at 94 °C for 30 s, 55–60 °C (depending on primers) for 30 s, 72 °C for 30–60 s, and a final extension at 72 °C for 7 min. Amplified products were separated by electrophoresis in agarose gels with ethidium bromide and photographed under ultraviolet light using the gel documentation system, or separated in 8–10 % denaturing polyacrylamide gel electrophoresis and observed by silver staining.

Development of new molecular markers

Based on Nipponbare reference sequences on NCBI, almost uniformly distributed sequences of the corresponding bacterial artificial chromosome (BAC) or P1-derived artificial chromosome (PAC) clones (http://www.tigr.org) between the markers 69B (Wang et al. 2006) and CP02662 (Wang et al. 2005) were selected for BLAST with all available rice genome sequences to find non-homologous or less homologous regions. Based on this analysis, 107 pairs of STS (sequence-tagged sites) marker primers were designed within this region. New primers were designed to generate PCR fragments ranging 100–1,000 bp in size.

Genetic and physical mapping of *Xa23*

The genetic map of *Xa23* was constructed according to genetic distances of the molecular markers linked to *Xa23* locus. Linkage analysis of the polymorphic markers was performed with MAPMAKER/EXP 3.0 (Lincoln et al. 1993). Recombination frequencies were converted to cM using Kosambi function (Kosambi 1944). The physical map was constructed by bioinformatically (http://blast.ncbi.nlm.nih.gov/) locating the linked makers on related BAC and PAC clones of Nipponbare.

Identification of putative candidate genes of *Xa23*

Based on the targeted region of *Xa23* locus, genomic sequence of Nipponbare between the markers Lj138 and A83B4 was downloaded from NCBI (http://www.ncbi.nlm.nih.gov) and analyzed using the online software FGENESH (http://linux1.softberry.com/). Candidate genes in the region of interest were identified by BLAST-Putility (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The *Xa23* candidates were finally determined by comparing the FGENESH predictions with those annotated by MSU Rice Genome Annotation Project (http://rice.plantbiology.msu.edu/).

Results

Resistance patterns of rice plants

Plants of two F_2 populations derived from the crosses of JG30/CBB23 and IR24/CBB23 were inoculated individually with *Xoo* strain PXO99 at booting stage. Since disease phenotypes of resistant and susceptible F_2 plants were clearly distinguishable, like their R and S parents (Fig. 1a), the lesion areas on the plant leaves were scored by visual assessment (Wang et al. 2005). For the F_2 population derived from JG30/CBB23, distribution of F_2 plants based on lesion area was bimodal, with an apparent valley

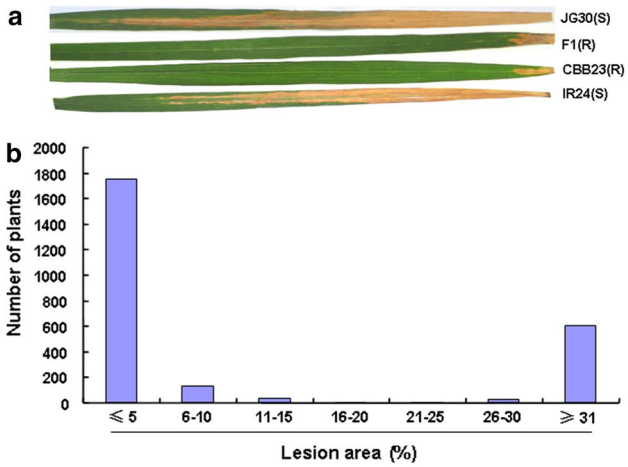

Fig. 1 Reaction patterns of rice plants to *Xanthomonas oryzae* pv. *oryzae* strain PXO99. **a** Leaves of parents JG30, CBB23, IR24 and the F_1 plants of cross JG30/CBB23 were presented to show the lesion patterns: *S* susceptible, *R* resistant. Pictures were taken 14-day post inoculation. **b** Distribution, based on the ratio of lesion area against the whole leaf area, of 2,562 F_2 plants derived from the cross JG30/CBB23. Lesion area (%) was scored 14-day post inoculation

at lesion area approximately 16–25 % (Fig. 1b). Inoculation assessment revealed that 1,930 plants of the F_2 population were resistant and 632 plants were susceptible; the R:S ratio fits 3:1 well ($\chi^2 = 0.13$, $P = 0.72$) (Table 1). Likewise, the R:S ratio of the F_2 population derived from IR24/CBB23 fits 3:1 perfectly ($\chi^2 = 0.03$, $P = 0.86$) (Table 1). These results indicated that the BB resistance in CBB23 was controlled by a single dominant gene.

High-resolution genetic and physical mapping of *Xa23*

In our previous investigations, the *Xa23* locus was located within a 1.7 cM region on long arm of rice chromosome 11 between molecular markers 69B (Wang et al. 2006) and CP02662 (Wang et al. 2005). The RFLP marker 69B was developed from the PAC clone 69B15 from indica rice Guangluai 4 (Wang et al. 2006). We then sequenced the ends of 69B and located it, by BLASTN (http://blast.ncbi.nlm.nih.gov/Blast.cgi), at nucleotide position 23846538-23848957 on chromosome 11 of japonica rice Nipponbare (NCBI Reference Sequence: NC_008404). CP02662 is an EST marker located at nucleotide position 24176326-24176528 on Nipponbare chromosome 11 (NC_008404) (Wang et al. 2005). We accordingly speculated that *Xa23* locus resides within a region corresponding to the 330-kb region (23846538-24176528 of NC_008404) of Nipponbare. To narrow down the region harboring the *Xa23* gene, 107 pairs of PCR primers were designed across the 330-kb sequences by searching the non- or low-homologous sequences. The surveyed results showed that

Table 2 STS markers developed in this study

Marker	Forward primer (5′–3′)	Reverse primer (5′–3′)	PCR product size (bp)		
			Nipponbare	JG30	CBB23
Lj36	gcaatggctagtaggaacga	atccgcacaagaacagtagc	295	No	1,150
Lj46	tatcagcttccatcccaagg	cgttaacagccaaccatcct	321	322	319
Lj138	acctccctcatcattcattcgt	ccaccgatttaaaagaaaatcgacggtg	190	190	194
Lj74	aagccatttgatgagcaacc	ggatccatttcagcataaccttt	869	869	983
A83B4	cttagtgcgtccagcctttc	tagcgcccctatatgtttgg	177	177	172
Lj13	gcactgcatgagagttggaa	gatgcaaccatagcccatct	435	No	437

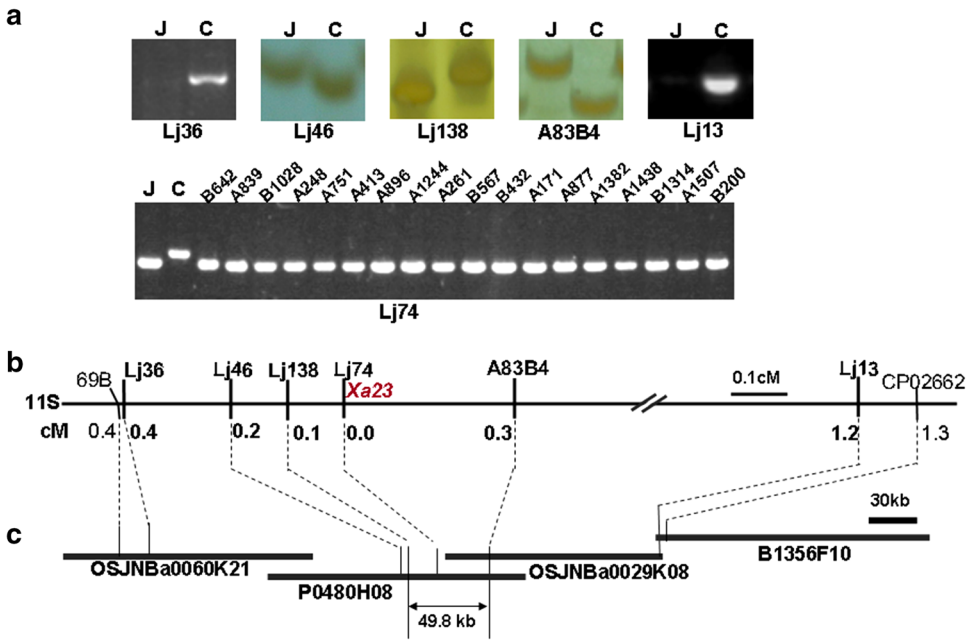

Fig. 2 Genetic and physical mapping of *Xa23* gene. **a** Polymorphisms between JG30 (J) and CBB23 (C) were revealed by STS markers Lj36, Lj46, Lj138, A83B4, Lj13, and Lj74. Molecular genotypes of some susceptible F_2 plants revealed by Lj74 were also shown. **b** Genetic map of *Xa23* locus. *Xa23* was mapped between the markers Lj138 and A83B4 on chromosome 11 (11S). The six new markers identified in this study, including the co-segregating maker Lj74, were shown in *bold*. **c** Physical map of *Xa23* locus. *Xa23* was located in a region corresponding to a 49.8 kb interval in the PAC clone P0480H08 of Nipponbare

six pairs of the designed primers (Table 2) revealed reliable polymorphisms between JG30 and CBB23 (Fig. 2a). The PCR products amplified from JG30 and CBB23 were sequenced to confirm the polymorphisms.

The six newly developed markers Lj36, Lj46, Lj138, Lj74, A83B4, and Lj13 (Table 2) were then used to survey the 632 susceptible and 167 resistant (randomly chosen) F_2 individuals derived from cross of JG30/CBB23. Results showed that Lj36, Lj46, Lj138, Lj74, A83B4, and Lj13 revealed 4, 3, 1, 0, 4, and 14 recombinant susceptible individuals, respectively (Table 3). Thus, the *Xa23* gene was defined to a 0.4 cM region between markers Lj138 and A83B4, in which the co-segregating marker Lj74 was identified (Table 3; Fig. 2b). To confirm this fine-mapping results, we used markers Lj46, Lj74 and A83B4 to survey all resistant and susceptible F_2 individuals derived from IR24/CBB23 (Table 1), and similar genetic map was obtained (data not shown).

The markers were then landed on the reference sequences of Nipponbare by bioinformatical analysis (Fig. 2c). Based on the pairwise BLAST analysis, the BACs and PACs were aligned as a contig map covering the *Xa23* locus. The markers Lj46, Lj138, Lj74, and A83B4 were landed on the same PAC clone P0480H08. The physical interval between Lj138 and A83B4 is about 49.8 kb (Fig. 2c).

Xa23 candidate genes

Recently, the quality of Nipponbare reference genome sequences has been improved (Sakai et al. 2013; Kawahara et al. 2013). Within the region flanked by the newly developed markers Lj138 and A83B4 (from 22182291 to 22232136) of the updated Os-Nipponbare-Reference-IRGSP-1.0 (http://rapdb.dna.affrc.go.jp/), nine genes have been annotated (http://rice.plantbiology.msu.edu/), including LOC_Os11g37650 that encodes the DWARF27 protein required for biosynthesis of strigolactones and thereby regulating rice tiller bud outgrowth (Lin et al. 2009) and two transposon protein-encoding genes (LOC_Os11g37590 and LOC_Os11g37600). The remaining six annotated genes are candidates of *Xa23* (Table 4). They

Table 3 Genotypes of F_2 recombinants revealed by closely linked molecular markers

F_2 plants	Phenotype	03STS	69B	**LJ36**	**Lj46**	**Lj138**	**Lj74**	**A83B4**	Lj13	CP02662	RM206
B642	Su	H	H	**H**	**H**	**H**	**S**	**S**	**S**	S	S
A839	Su	H	H	**H**	**H**	**S**	**S**	**S**	**S**	S	S
B1028	Su	H	H	**H**	**H**	**S**	**S**	**S**	**S**	S	S
A248	Su	H	H	**H**	**S**	**S**	**S**	**S**	**S**	S	S
A751	Su	H	S	**S**	**S**	**S**	**S**	**S**	**S**	S	S
A413	Su	S	S	**S**	**S**	**S**	**S**	**S**	**S**	S	H
A896	Su	S	S	**S**	**S**	**S**	**S**	**S**	**S**	S	H
A1244	Su	S	S	**S**	**S**	**S**	**S**	**S**	**S**	S	H
A261	Su	S	S	**S**	**S**	**S**	**S**	**S**	**S**	H	H
B567	Su	S	S	**S**	**S**	**S**	**S**	**S**	**S**	H	H
B432	Su	S	S	**S**	**S**	**S**	**S**	**S**	**H**	H	H
A171	Su	S	S	**S**	**S**	**S**	**S**	**S**	**H**	H	H
A877	Su	S	S	**S**	**S**	**S**	**S**	**S**	**H**	H	H
A1382	Su	S	S	**S**	**S**	**S**	**S**	**S**	**H**	H	H
A1438	Su	S	S	**S**	**S**	**S**	**S**	**S**	**H**	H	H
B1314	Su	S	S	**S**	**S**	**S**	**S**	**S**	**H**	H	H
A1507	Su	S	S	**S**	**S**	**S**	**S**	**S**	**H**	H	H
B200	Su	S	S	**S**	**S**	**S**	**S**	**S**	**H**	H	H
A64	Su	S	S	**S**	**S**	**S**	**S**	**S**	**H**	H	R
B548	Su	S	S	**S**	**S**	**S**	**S**	**S**	**H**	H	R
A1480	Su	S	S	**S**	**S**	**S**	**S**	H	**H**	H	H
B198	Su	S	S	**S**	**S**	**S**	**S**	**H**	**H**	H	H
B592	Su	S	S	**S**	**S**	**S**	**S**	**H**	**H**	H	H
B1025	Su	S	S	**S**	**S**	**S**	**S**	**H**	**H**	H	H
B176	Re	H	H	**H**	**H**	**H**	**H**	**R**	**R**	R	R
B188	Re	H	H	**H**	**H**	**H**	**H**	**R**	**R**	R	R
A123	Re	R	R	**R**	**R**	**R**	**R**	**R**	**R/H**	R/H	H
B148	Re	R	R	**R**	**R**	**R**	**R**	**R**	**R/H**	R/H	H
A132	Re	H	H	**H**	**H**	**H**	**H**	**H**	**H**	H	S

The five resistant recombinants were identified from 167 randomly chosen resistant F_2 plants

Su susceptible phenotype, *Re* resistant phenotype, *S* homozygous genotype of JG30, *R* homozygous genotype of CBB23, *H* heterozygous genotype from parents JG30 and CBB23, *R/H* genotype either R or H, *03STS* converted STS marker from the RFLP marker C1003A (Fan et al. 2006)

Six new markers developed in this study are shown in bold

Table 4 Predicted candidate genes of *Xa23* (http://rice.plantbiology.msu.edu/)

Gene	Nucleotide position[a]	Locus[b]	Protein encoded
1	22187684–22186464	LOC_Os11g37580	Hypothetical protein, 218 aa
2	22202646–22199410	LOC_Os11g37610	Hypothetical protein, 230 aa
3	22204676–22203734	LOC_Os11g37620	Hypothetical protein, 164 aa
4	22212647–22208279	LOC_Os11g37630	Hypothetical protein, 837 aa
5	22215791–22219121	LOC_Os11g37640	Putative ADP-ribosylation factor-like protein 5, 185 aa
6	22231042–22223651	LOC_Os11g37660	Putative vegetative storage protein, PPR (pentatricopeptide repeat) domain, 678 aa

[a] TSS → PolyA

[b] MSU Rice Genome Annotation Project http://rice.plantbiology.msu.edu/)

encode four hypothetical proteins (LOC_Os11g37580, LOC_Os11g37610, LOC_Os11g37620 and LOC_Os11g37630), a putative ADP-ribosylation factor protein (LOC_Os11g37640) and a putative vegetative storage protein (LOC_Os11g37660).

Discussion

The *Xa23* locus in CBB23 is originally from wild rice *O. rufipogon* (Zhang et al. 2002). Studies in the past decade repetitively verified that the single *Xa23* locus confers high

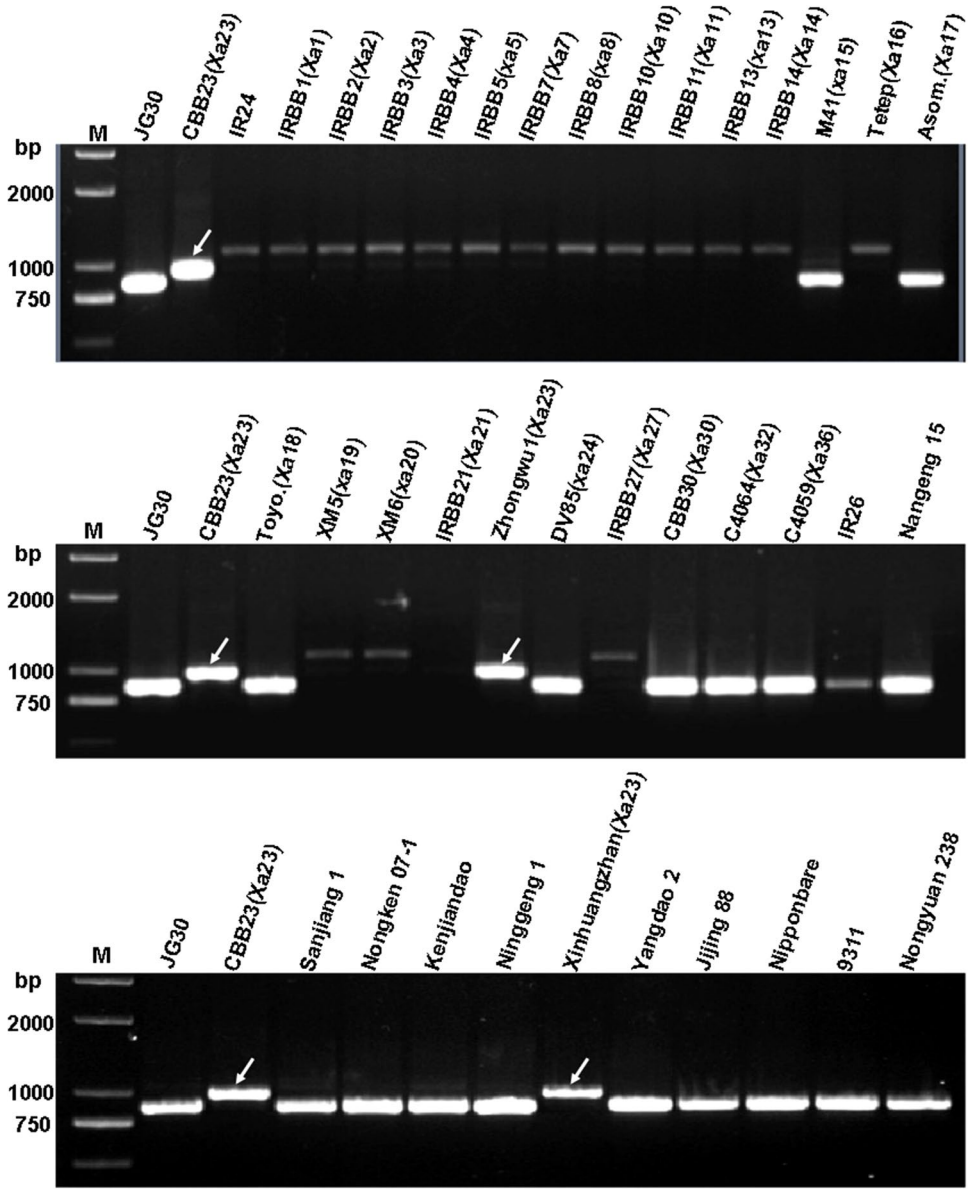

Fig. 3 Polymorphism assays of 39 rice varieties/lines using the co-segregating maker Lj74. The specific 983-bp bands amplified only from *Xa23*-containing varieties/lines were indicated by *arrows*. *M* molecular ladder

resistance to more than 30 representative *Xoo* strains from China, Philippines, Japan, Korea, and Bangladesh. In fact, no naturally occurring *Xoo* isolate that can overcome the *Xa23*-mediated resistance has been identified so far (Wang et al. 2013). Furthermore, *Xa23* locus confers dominant BB resistance at all growth stages, an important feature for hybrid rice breeding (Zhang et al. 2002). Thus, CBB23 has been widely adopted in rice breeding programs in China (Zhou et al. 2009; Huang et al. 2012). In this study, we identified six new STS markers co-segregated with or closely linked to the *Xa23* locus and mapped the *Xa23* gene within a 0.4 cM region, corresponding to a 49.8 kb physical distance on Nipponbare genome, in which 6 *Xa23* candidate genes have been annotated. The high-resolution genetic map of *Xa23* locus and the co-segregating or closely linked markers will certainly facilitate both marker-assisted selection and molecular cloning of *Xa23*.

Among the six STS markers developed in this study, Lj36 and Lj13 are dominant markers, generating amplicons in the *Xa23*-donor parent CBB23 but not in the recipient parent JG30 (Fig. 2a). Lj46, Lj138, and A83B4 are co-dominant markers, but polyacrylamide gel electrophoresis must be adopted to differentiate the CBB23- and JG30-amplicons due to their very small (3–5 bp) differences in size (Table 2; Fig. 2a). Comparatively, Lj74 should be the most effective marker for selection of *Xa23*, because it is a co-segregating and co-dominant marker, generating CBB23- and JG30-amplicons with 114 bp difference in size (Table 2), clearly differentiated in agarose gel (Fig. 2a). We used Lj74 to survey the polymorphisms of 36 additional

rice varieties/lines, including 24 lines harboring different BB resistance genes. The results showed that the specific 983-bp band can be amplified only from *Xa23*-containing varieties/lines (Fig. 3), indicating that Lj74 would be very useful in rice breeding for the marker-assisted selection of *Xa23*.

Among the six candidates of Xa23, LOC_Os11g37580 is a hypothetical protein with no functional domain predicted based on its amino acid sequences. The gene is highly conserved in rice, poplar, *Brachypodium*, maize and sorghum, but its biological function is unclear. LOC_Os11g37610 is also a hypothetical protein with a transmembrane region. Orthologous genes exist in rice but not in other organisms. LOC_Os11g37620 is another hypothetical protein with three transmembrane regions. No orthologous gene has been found. LOC_Os11g37630 is a hypothetical protein with high identity to uncharacterized leaf senescence protein-like proteins of rice, conserved in poplar, *Arabidopsis*, *Brachypodium*, maize, grapevine and sorghum. LOC_Os11g37630 contains a transmembrane region and two PMR5 domains. Plant proteins with PMR5 have a C-rich sugar binding domain followed by the PC-Esterase (acyl esterase) domain. Plant proteins with PMR5 may play important roles in host–pathogen interactions, regulation of transpiration and stress resistance (Xin et al. 2007; Anantharaman and Aravind 2010). LOC_Os11g37640 is an ADP-ribosylation factor (ARF)-like protein with a signal peptide at *N*-terminus. ARF proteins are conserved in various organisms. In plants, ARF proteins have been reported to play roles in controlling cell cycle during seed development, intracellular signaling, and membrane trafficking (Matheson et al. 2007; Cevher-Keskin 2013), associated with endocytosis in plant cells (Naramoto et al. 2010) and replication of red clover necrotic mosaic virus, a plant RNA virus (Hyodo et al. 2013). The relationship between ARF proteins and plant disease resistance has been established (Lee et al. 2003b; Lee and Sano 2007; Böhlenius et al. 2010; Nielsen et al. 2012). Rice ARF protein has been demonstrated to be involved in fungal disease response (Lee et al. 2003b). LOC_Os11g37660 is a putative vegetative storage protein, containing nine tandem pentatricopeptide repeats (PPRs). PPR proteins are eukaryote-specific RNA-binding proteins, involved in multiple aspects of RNA metabolism of organellar genes (Nakamura et al. 2012). Recent investigation has shown that the PPR protein PPR8522 is necessary for maize embryogenesis and vegetative development (Sosso et al. 2012). PPR proteins usually act in a gene-specific manner (Nakamura et al. 2012; Härtel et al. 2013). So far, no one has reported that a PPR protein is involved in plant disease resistance.

We have cloned the cognate avr-gene of *Xa23* from *Xoo* strain PXO99 (GenBank: GU732172.1). The *avrXa23* encodes a member of transcription activator-like (TAL) effectors (Wang et al. 2013). It has been demonstrated that *Xa23*-dependent BB resistance was resulted from a classical gene-for-gene interaction between CBB23 and *Xoo* strains (Wang et al. 2013). Thus, we speculate that *Xa23* should be a member of the so-called executor type *R* genes whose expressions are activated by TAL effectors. Among the cloned dominant BB resistance genes, only *Xa10* (Tian et al. 2014) and *Xa27* (Gu et al. 2005) belong to the executor type *R* genes. Notably, recent work revealed that the conserved domains of Xa10 are highly homologous with the hypothetical protein (ABA94457) deduced from LOC_Os11g37620, even if their nucleotide sequences are largely different (Tian et al. 2014). Therefore, LOC_Os11g37620 is most likely the candidate of *Xa23*. However, LOC_Os11g37620 presents in Nipponbare that lacks the *Xa23*-mediated BB resistance. Thus, we speculate that the *Xa23* might encode a protein different from ABA94457. Accomplishment of molecular cloning of *Xa23* would confirm this speculation.

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