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ORIGINAL PAPER

Xa3, conferring resistance for rice bacterial blight and encoding a receptor kinase-like protein, is the same as *Xa26*

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Abstract *Xa3*-mediated resistance for rice bacterial blight, one of the most devastating rice diseases worldwide, is influenced by genetic background. *Xa3* is genetically tightly linked to *Xa26*, another gene for bacterial blight resistance. *Xa26* belongs to a clustered multigene family encoding leucine-rich repeat (LRR) receptor kinase-like proteins. To characterize *Xa3*, we fine mapped it using a population segregating for only one resistance gene and markers developed from *Xa26* family. Genetic analysis showed that *Xa3* co-segregated with the marker of *Xa26* gene and segregated from the markers of other members of *Xa26* family. DNA fingerprinting revealed that rice line IRBB3 carrying *Xa3* had the same copy numbers of *Xa26* family members as rice line Minghui 63 carrying *Xa26*. Phenotypic comparison showed that all the rice lines carrying either *Xa3* or *Xa26* developed dark brown deposition at the border between the lesion caused by incompatible-pathogen infection and health leaf tissue, while other rice lines did not show this dark brown deposition in either incompatible or compatible interactions. These results suggest that *Xa3* and *Xa26* is the same gene. We name it *Xa3/Xa26* to indicate the relationship between the two gene symbols. The putative encoding products of *Xa3/Xa26* and its susceptible allele *xa3/xa26* shared 92% sequence identity. The sequence difference occurred in the LRR domains, specifically at the solvent-exposed amino acid residues, might be the major cause that differentiates the resistant and susceptible proteins.

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Introduction

Bacterial blight, caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), is one of the most serious rice diseases in the world. In total, 30 genes conferring host resistance against various strains of *Xoo* have been identified and these genes have been designated in a series from *Xa1* to *Xa29* (Lin et al. 1996; Nagato and Yoshimura 1998; Zhang et al. 1998; Khush and Angeles 1999; Chen et al. 2002; Lee et al. 2003; Yang et al. 2003; Tan et al. 2004). Several of the resistance (*R*) genes have been assigned with the same symbol by different researchers, such as the dominant *R* gene *Xa26* and recessive *R* gene *xa26* (Lee et al. 2003; Yang et al. 2003), and two genes named as *Xa25* (Gao et al. 2001; Chen et al. 2002). In addition, a few *R* genes have been assigned with two or more symbols. For example, *Xa3* have also been named as $Xa4^b$, *Xa6*, and *xa9* (Sidhu and Khush 1978; Ogawa et al. 1986a, b, c, 1988a). Only six of the 30 genes, *Xa1*, *xa5*, *xa13*, *Xa21*, *Xa26*, and *Xa27*, have been reported to be isolated. *Xa21* and *Xa26* encode leucine-rich repeat (LRR) receptor kinase-like proteins (Song et al. 1995; Sun et al. 2004). *Xa1* encodes a nucleotide binding-LRR protein (Yoshimura et al. 1998). The recessive gene *xa5* encodes the gamma subunit of transcription factor IIA (Iyer and McCouch 2004). *Xa27* encodes a novel protein (Gu et al. 2005). The fully recessive gene *xa13* encodes a novel plasma membrane protein (Chu et al. 2006).

The *Xa3* gene was first identified in a *japonica* variety Wase Aikoku 3 and designated as *Xa-w* (Ezuka et al. 1975), which was late named as *Xa3* (Ogawa 1987). *Xa3* has a relative wide resistance spectrum. Plants carrying *Xa3* are resistant to six (races 1, 2, 3, 4, 5, and 9) of the nine Philippine *Xoo* races (Ogawa et al. 1986b; Zhang et al. 1998). *Xa3* is mapped to the long arm of rice chromosome 11 and tightly linked to another bacterial blight resistance gene *Xa4* (Yoshimura et al. 1992, 1995). The function of *Xa3* is influenced by both genetic background and growth stage of host, which results in variable resistant spectrum and dominant or recessive resistance in *Xa3*-mediated defense (Sidhu and Khush 1978; Ogawa et al. 1986a, b, 1986c, 1988a). However, the mechanisms of the features of *Xa3*-mediated resistance are unknown, which leads to applicable difficulty of improving rice resistance using this gene.

The *Xa26* gene was first identified in an *indica* rice cultivar Minghui 63 and mapped to rice chromosome 11 (Yang et al. 2003), where loci of other bacterial blight resistance genes, *Xa3*, *Xa4*, and *Xa22(t)*, were tightly linked (Yoshimura et al. 1995; Lin et al. 1996). In addition to similar chromosomal location as *Xa3*, the function of *Xa26* is also influenced by genetic background (Sun et al. 2004). Transgenic plants carrying *Xa26* in a *japonica* background show enhanced resistance compared with Minghui 63. Minghui 63 is the restorer line for a number of rice hybrids that collectively have been planted for more than 20% of the total rice production area in China during the last two decades. The hybrids produced with Minghui 63 have a number of desirable characteristics including high yielding and wide adaptability. One of the important characteristics accounting for the wide adaptability of the hybrids produced with Minghui 63 is their resistance to *Xoo*. Thus, *Xa26* is a durable resistance gene in rice production at least in China. Further study showed that two other rice lines, IRBB3 carrying presumably only one *R* gene, *Xa3*, for bacterial blight resistance (Ogawa et al. 1991) and Zhachanglong (GenBank accession DQ426646) carrying *Xa22(t)* (Lin et al. 1996), also carried *Xa26* gene as revealed by comparative sequence analysis (Sun et al. 2004). *Xa26* belongs to a multigene family. The four members of *Xa26* family cluster in tandem (Sun et al. 2004). These results raise the question whether *Xa3* also encodes a LRR receptor kinase-like protein or another type protein.

Characterization of *Xa3* is the first step to understand the molecular mechanism of *Xa3*-mediated resistance. To answer the above question, we fine mapped *Xa3* using molecular markers developed from *Xa26* family. The results show that *Xa3* and *Xa26* designated for the *R* genes in different rice lines are actually the same gene according to the results of fine genetic mapping, DNA fingerprinting and phenotypic comparison of different rice lines carrying different *R* genes for bacterial blight resistance. This gene is now also named as *Xa3/Xa26* to indicate the relationship between the two gene symbols. *Xa3/Xa26* encodes a LRR receptor kinase-like protein. Both *Xa3/Xa26* and its susceptible allele *xa3/xa26* are constitutively expressed. The resistant protein XA3/XA26 and susceptible protein *xa3/xa26* have 8% sequence divergence, which results in different functions of the two proteins in disease resistance.

Materials and methods

Xoo inoculation and disease evaluation

At the booting stage, five of the uppermost fully expanded leaves of each plant were inoculated with Philippine race 1 (PXO61), race 2 (PXO86) or race 6 (PXO99) of *Xoo* by leaf-clipping method (Kauffman et al. 1973) in a disease nursery. The preparation of bacterial inoculum was as described by Lin et al. (1996). Reaction to the pathogen was evaluated three weeks after inoculation by measuring the lesion length (cm) of three leaves.

Fine genetic mapping of *Xa3*

An F_2 population was used to determine the relationship of bacterial blight resistance genes *Xa3* and *Xa26* by fine genetic mapping of *Xa3* using the probes of *Xa26* gene family. This segregation population consisted of about 2,000 F_2 individuals from a cross between a resistance rice line IRBB3 (*Oryza sativa* ssp. *indica*) carrying *Xa3* and its susceptible near-isogenic parental line IR24 (*O. sativa* ssp. *indica*) (Ogawa et al. 1986c, 1988b, 1991). IRBB3 is resistant to *Xoo* strain PXO86 and rice cultivar Minghui 63 carrying *Xa26* gene is moderately susceptible to PXO86 (Ogawa et al. 1986c; Yang et al. 2003; Sun et al. 2004). To evaluate the relationship of *Xa3* and *Xa26*, *Xoo* strain PXO86 was chosen to study the interaction of IRBB3 and *Xoo*.

Two restriction fragment length polymorphism (RFLP) markers, R1506 and S10559 commonly used by rice community, linked to *Xa26* gene (Sun et al. 2004) and three RFLP markers of *Xa26* gene family, 2/15B-29, M196-1 and Rkc developed in our lab, were used to screen the 317 highly susceptible individuals from the population. Markers 2/15B-29 and M196-1

were the subclones of *RKa*, a member of *Xa26* family, and *Xa26* gene, respectively (Sun et al. 2004). Marker Rkc, another member of *Xa26* family, was obtained by amplification of genomic DNA of rice cultivar Minghui 63 through polymerase chain reaction (PCR) using primers RKc-L (5′-TGTTTCGAGTGGCATACAGC-3′) and RKc-R (5′-ATGAGCCGAGCAATGATACC-3′) (Sun et al. 2004).

Determining the sequence of susceptible allele x*a3/xa26*

PCR method was used to isolate overlapped fragments of the susceptible allele of *Xa3/Xa26*, *xa3/xa26*, from rice line IR24 using primers designed according to the sequences of *Xa26* (Sun et al. 2004) and *xa3/xa26*. The primers based on *Xa26* in rice line Minghui 63 were Mkb-F (5′-ATGGCTCTTGTTCGATTGCC-3′), Rkb-L (5′-GGCTTGCAAACTTTGGACAT-3′), Rkb-R (5′-GCTTCCCTTGTTCTGAGTGC-3′), Rkb-4F (5′-AGCGATGATAGCATGTTGGG-3′), Rkb-3R (5′-TCAGTGTCAAGACCACATCG-3′), and Tkb-R (5′-ACTGCTGCACAGCGTTC TC-3′). The primers based on the susceptible allele *xa3/xa26* in rice line IR24 were TA1F (5′-GCCCT ATCCCTGGTAATAC A-3′), TA1R (5′-TTGCACGTCGACAAATCTAA-3′), TA2F (5′-TGCAATTCCAGAATCAATCA-3′), and TA2R (5′-TTCAAGCTAACAAGGGTCGT-3′).

Overlapped transcript fragments of *xa3/xa26* were obtained by reverse transcription (RT)-PCR. Total RNA was treated with DNase I (Invitrogen Life Technologies, Carlsbad, California, USA) to remove contaminating DNA and used for RT-PCR in a two-step reaction (Zhou et al. 2002). In brief, the RT step was performed in a 10 µl volume that contained 0.5–1 µg total RNA pretreated with DNase I, 50 ng primer, 100 U M-MLV reverse transcriptase (Promega Corporation, Madison, WI, USA), 1 X first-strand buffer (50 mmol/l Tris–HCl [pH 8.3], 75 mmol/l KCl, and 3 mmol/l $MgCl_2$), 20 mmol/l DTT, and 10 µmol/l each of dATP, dCTP, dGTP, and dTTP at 42°C for 1.5 h. The mixture was diluted by the addition of 40 µl of deionized water after the RT reaction, and 1 µl of the diluted mixture was used for PCR. The oligo $(dT)_{15}$ and gene-specific primer 5′ race (5′-ATCAACCGG CA-3′) were used for RT. The PCR primers were Mkb-F, TA1F, TA2F, TA2R, Rkb-L, S1 (5′-CGTAG AACTGGGAGGCTGAA-3′), and Rkb2R (5′-CA GTCCACCACATGGACAAG-3′).

The genomic fragments and products of RT-PCR were directly sequenced using the gene-specific primers listed above or cloned into pGEM-T vector (Promega Corporation, Madison, USA) and then sequenced using M13 universal forward and reverse primers. Sequences were assembled using the computer program SEQUENCER 4.1.4 (Gene Codes Corporation, Ann Arbor, MI, USA).

Results

Segregation of resistance

In the present experimental condition, IRBB3 was highly resistant to PXO86 with an average lesion length of 1.0 ± 0.50 cm and IR24 was highly susceptible to PXO86 with an average lesion length of 20.3 ± 4.79 cm at three weeks after inoculation. Two hundred and sixty four F_2 individuals, randomly chosen from the mapping population developed from the cross between IRBB3 and IR24, were used to study the distribution of the lesion length caused by the infection of PXO86. The distribution of lesion length was bimodal with an apparent valley at approximate 7–9 cm (Fig. 1). Using a lesion length of 9 cm as the dividing point, the numbers of resistant (lesion length ≤9 cm) and susceptible (lesion length >9 cm) individuals were 202 and 62, respectively, which fit the expected 3:1 ratio (χ^2=0.25, $P > 0.5$). This result suggests that the resistance of IRBB3 to PXO86 was controlled by one dominant gene.

Evaluation of the relationship of *Xa3* and *Xa26*

The *Xa26* gene family consists of four members, *Xa26* or *RKb*, *RKa*, *RKc*, and *RKd*, clustered in tandem in a 40 kb region of rice cultivar Minghui 63; comparative sequence analysis showed that the alleles of *Xa26* and *RK*a in IRBB3 were identical to those in Minghui 63, indicating that IRBB3 carries *Xa26* gene (Sun et al. 2004). Further comparing the sequences flanking *Xa26*

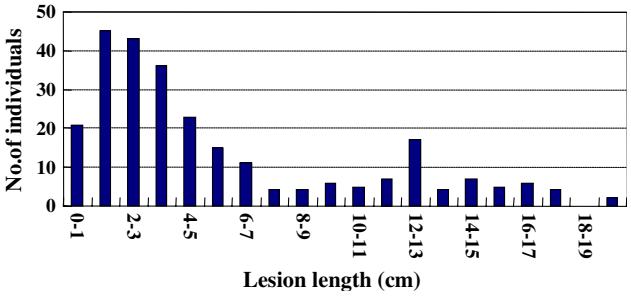

Fig. 1 Distribution of lesion length in a sample containing 264 individuals from a F_2 population segregating for *Xa3* gene. The plants were inoculated with *Xoo* strain PXO86

in IRBB3 (GenBank accession DQ426645) and Minghui 63 (GenBank accession DQ355952) revealed one nucleotide-substitution occurring at 475 bp in front of translation initiation site and 12 nucleotide-substitutions at 602–624 bp region following the 3′ untranslated region of *Xa26*. Two commonly used RFLP markers, R1506 and S10559 tightly linked to *Xa26*, and three markers, 2/15B-29, M196-1 and Rkc, of *Xa26* family detected polymorphism between the parents of the mapping population, IRBB3 and IR24 (Fig. 2). These markers were used to screen the 317 highly susceptible individuals, which had the lesion length ranged from 9.5 to 27.5 cm, from the F_2 population. The results showed that *Xa3* was flanked by markers R1506 and S10559 (Fig. 3). Five F_2 individuals (2-1, 3-105, 2-10, 3-143, and 3-186) from the 317 highly susceptible plants showed recombination events between the *Xa3* locus and the flanking marker R1506 (Figs. 2, 3). Another five susceptible F_2 plants (3-181, 3-79, 3-85, 3-202, and 3-216) showed recombination events between the *Xa3* locus and another flanking marker S10559 (Figs. 2, 3). The markers of *Xa26* family were further used to examine the 10 recombinants (Table 1). Marker 2/15B-29 of *RKa* gene detected three recombination events, placing itself 0.5 cm from *Xa3* locus (Figs. 2, 3). Marker Rkc from *RKc* gene identified one

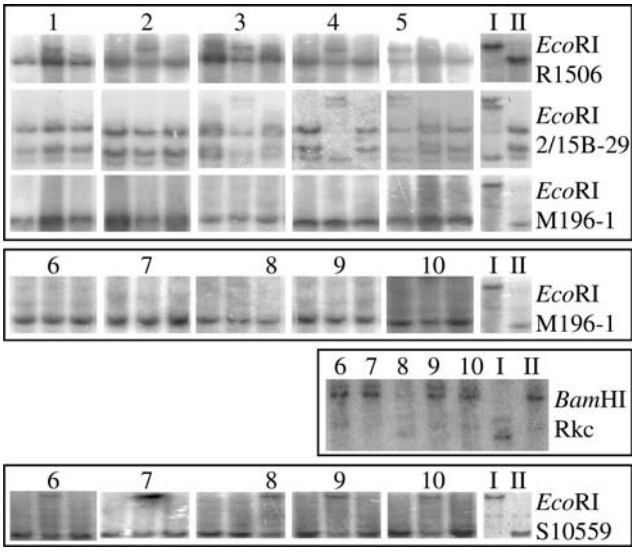

Fig. 2 Southern-blot analysis of total DNA from the parents, resistant IRBB3 (I) and susceptible IR24 (II), and the highly susceptible F_2 individuals of a F_2 population segregating for *Xa3* gene. The DNA was digested with either *Eco*RI or *Bam*HI, and hybridized with different probes. The hybridization patterns of the ten recombinant F_2 individuals (labeled with fingers) and their neighbor F_2 individuals (unlabeled) on nylon filters detected by each polymorphic probe are presented. The recombinant F_2 individuals are 2–1 (1), 3–105 (2), 2–10 (3), 3–143 (4), 3–186 (5), 3–79 (6), 3–85 (7), 3–181 (8), 3–202 (9), and 3–216 (10)

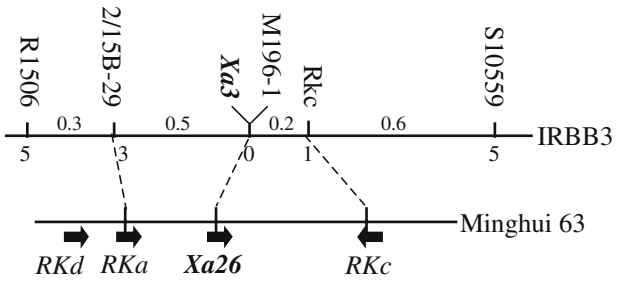

Fig. 3 A map covering the *Xa3/Xa26* region in rice lines IRBB3 and Minghui 63. The upper part is the genetic map of *Xa3* in IRBB3. The upper figures are the genetic distance between molecular markers or between molecular marker and *Xa3* locus. The bottom figures are the number of recombination events between the *Xa3* locus and the markers detected using 317 highly susceptible plants from a F_2 population segregating for *Xa3*. The lower part is the physical map of *Xa26* gene family in Minghui 63 (Sun et al. 2004). The *horizontal arrows* indicate the positions and transcription orientations of *Xa26* family members. The *dashed lines* indicate the positions of the markers relative to the members of *Xa26* family

recombinant, which was different from the three recombinants detected by 2/15B29 (Table 1), placing itself 0.2 cm from *Xa3* on another side of the *Xa3* locus (Fig. 3). The *Xa3* co-segregated with marker M196-1 of *Xa26* gene in the 10 plants. These results suggest that *Xa3* and *Xa26* may be the same gene.

To verify the inference that *Xa3* and *Xa26* are the same gene, the following three approaches were applied. First, the marker of *Xa26* gene, M196-1, was used to screen the 264 F_2 individuals (Fig. 1) randomly chosen from the mapping population to examine the relationship between marker genotypes and resistance. The result showed that all the 199 plants with lesion length shorter than 9.0 cm were either homozygous for the IRBB3 allele or heterozygote at *Xa26* locus. Whereas all the 58 plants with lesion length longer than 9.5 cm were homozygous for the IR24 allele, only seven plants had lesion length between 9.0 and 9.5 cm; four of them were homozygous for the IR24 allele and the other three were heterozygote. Thus the segregation of *Xa26* genotype agrees well with the resistance in this population.

Second, the copy number of *Xa26* family members in rice line IRBB3 was evaluated. The coding regions of *Xa26* family members (GenBank accession DQ355952), *Xa26*, *RKa* and *RKc*, share 62–78% sequence identity in rice variety Minghui 63 (Sun et al. 2004). The probes of the three members of *Xa26* family were used to examine the copy numbers of the homologous genes in different rice lines. Rice lines IRBB3 and Zhachanglong showed a similar DNA fingerprint pattern to Minghui 63, suggesting that the two rice lines carry the same numbers of *Xa26* family member as Minghui 63 (Fig. 4). Thus there should be no other

Table 1 Molecular marker genotypes of ten susceptible F_2 recombinant individuals

Marker	Individuals[a]									
	2–1	3–105	2–10	3–143	3–186	3–181	3–79	3–85	3–202	3–216
R1506	H	H	H	H	H	S	S	S	S	S
2/15B-29	S	S	H	H	H	S	S	S	S	S
M196–1	S	S	S	S	S	S	S	S	S	S
Rkc	S	S	S	S	S	H	S	S	S	S
S10559	S	S	S	S	S	H	H	H	H	H

[a] S and H stand for the homozygous susceptible and heterozygous genotypes, respectively, of the F_2 recombinants from the cross between the resistant and susceptible near-isogenic parents, IRBB3 and IR24.

Xa26 family member locating between *Xa3* locus and marker 2/15B-29, M196-1 or Rkc (Fig. 3).

Thirdly, the lesion appearance of different rice lines that were known to carry *Xa3* or *Xa26* upon infection was compared. It has been known that rice lines carrying *Xa3* gene present dark brown deposition at the border between the lesion and health leaf tissue after incompatible-*Xoo* infection (Kaku et al. 1978). Four rice lines, Zhachanglong, Minghui 63, Rb17-2 and IRBB3 carrying *Xa26* gene and a set of near-isogenic rice lines, IRBB3, IRBB4, IRBB10, IRBB13, IRBB21, and IR24, carrying *Xa3* or other *R* genes against *Xoo* or being susceptible to *Xoo* and having the same genetic background, were inoculated with incompatible or compatible-*Xoo* strain. The four *Xa3*- or *Xa26*-carrying lines, Zhachanglong, Minghui 63, Rb17-2 and IRBB3, all developed the dark brown deposition on the border between the lesion and health leaf tissue (Fig. 5). Whereas no such brown border deposition was observed in other rice lines either carrying at least one of other four *R* genes for *Xoo* resistance or being susceptible to *Xoo*. According to the lesion feature, the correlation of resistance and *Xa26*-genotype, DNA fingerprint and the result of fine genetic mapping, it can be confirmed that *Xa3* and *Xa26* are the two symbols for one gene. This gene is described as *Xa3/Xa26* in the following text.

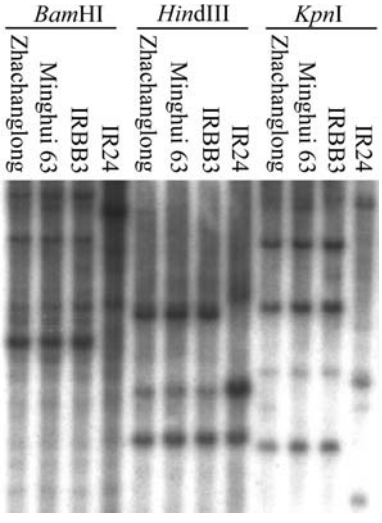

Fig. 4 Southern-blot analysis of the total DNA from rice lines Zhachanglong, Minghui 63, IRBB3 and IR24. The DNA was digested with *Bam*HI, *Hin*dIII, or *Kpn*I and hybridized with a mixed probe of 2/15B-29, M196–1 and Rkc

The susceptible allele *xa3/xa26*

Comparative analysis of the genomic and cDNA sequences of *xa3/xa26* from susceptible rice line IR24 (GenBank accession DQ426647) showed that the coding region of the gene was 3,300 bp in length and interrupted by one intron of 106 bp. The coding region of *xa3/xa26* shared 96% sequence identity with that of *Xa3/Xa26* from resistant rice lines IRBB3 and Minghui 63 (Sun et al. 2004). The putative encoding product of *xa3/xa26* consisted of 1,100 amino acids as compared to 1,103 amino acids encoded by the resistance *Xa3/Xa26*. The susceptible and resistant proteins shared 92% sequence identity and 94% sequence similarity (Fig. 6). According to the domain alignment of the resistant protein (Sun et al. 2004), xa3/xa26 and XA3/XA26 had sequence divergence, which occurred in all the characterized structures of a LRR-receptor kinase protein, including signal peptide, LRR domain, transmembrane region and kinase domain. Approximate half (41) of the divergent sites between the susceptible and resistant proteins occurred in the kinase domain (Fig. 6). In addition, there were 25 divergent sites with eight being solvent-exposed amino acid residues in the LRR domain.

Discussion

The present results suggest that the genes, designated as *Xa3* and *Xa26* in rice lines IRBB3 and Minghui 63, respectively, are actually the same gene. We suggest naming it *Xa3/Xa26* to indicate the relationship the two gene symbols. The following evidences support

Fig. 5 Leaves from different rice lines at 3 weeks after inoculation with *Xoo* strains PXO61, PXO86 or PXO99. The IRBB3, IRBB4, IRBB10, IRBB13 and IRBB21 are *indica* near-isogenic lines known to carry bacterial blight resistance genes *Xa3*, *Xa4*, *Xa10*, *xa13,* and *Xa21*, respectively (Ogawa et al. 1991). These near-isogenic lines all have the genetic background of IR24 that is susceptible to *Xoo*. IRBB3, *indica* rice cultivar Minghui 63 (MH63), *japonica* rice Zhachanglong (ZCL) and transgenic rice line Rb17-2 that has the genetic background of *japonica* rice Mudanjiang 8 (MDJ8) carry *Xa26* gene (Sun et al. 2004). Mudanjiang 8 is susceptible to *Xoo*. All the rice lines carrying either *Xa3* gene or *Xa26* gene show dark brown deposition (*arrows*) locating at the border between the lesion and health tissue after being inoculated with incompatible pathogen PXO61 or PXO86

this conclusion. First, genetic analysis further confirmed that a single dominant gene mediated the resistance of IRBB3 to Xoo. IRBB3 is known to contain only one *R* gene, named as *Xa3*, for bacterial blight resistance (Ogawa et al. 1991). Thus, the gene mapped in this study should be *Xa3*. The *Xa3* locus co-segregated with M196-1, the marker of *Xa26* gene. The other genes of *Xa26* family, *RKa* and *RKc* that were tightly linked to *Xa26* from two sides, segregated from *Xa3* locus. DNA fingerprinting indicates that IRBB3 does not carry additional copies of *Xa26* family members compared with Minghui 63. Since sequence analysis has revealed that IRBB3 carries both *Xa26* and *RKa* (Sun et al. 2004), the resistance locus co-segregated with M196-1 in IRBB3 should be *Xa26*. In other words, *Xa3* and *Xa26* represent one gene.

Second, rice lines carrying either *Xa3* or *Xa26* showed the same lesion appearance in the interaction with *Xoo*. It had been reported that rice lines carrying *Xa3* developed dark brown deposition at the border between the lesion caused by incompatible-*Xoo* infection and the health leaf tissue (Kaku et al. 1978). The present results showed that all the four rice lines carrying *Xa3* or *Xa26* presented this type of phenotypic reaction. However, no such dark brown deposition at the border of lesion was observed in other rice lines having the same genetic background as those carrying *Xa3* or *Xa26* in either incompatible or compatible interactions.

Lastly, the function of the gene designated either as *Xa3* or *Xa26* in different rice varieties is apparently influenced by genetic background. *Xa3* is the most misapprehended resistance gene in the literature of rice bacterial blight studies. It behaved very differently in different genetic backgrounds and different growth stages, and it even displayed dominance reversal in one case (Sidhu and Khush 1978; Ogawa et al. 1986a, b, 1986c, 1988a). That is why several symbols, $Xa4^b$, *Xa6,* and *xa9*, have been assigned to this gene. *Xa26* gene also showed different resistance spectrum in different rice lines, including its original carriers, *indica* rice lines Minghui 63 and IRBB3 as well as *japonica* rice Zhachanglong, and *Xa26*-transgenic line (Rb17-2) with the background of *japonica* rice Mudanjiang 8 (Yang et al. 2003; Sun et al. 2004). In addition to the difference of

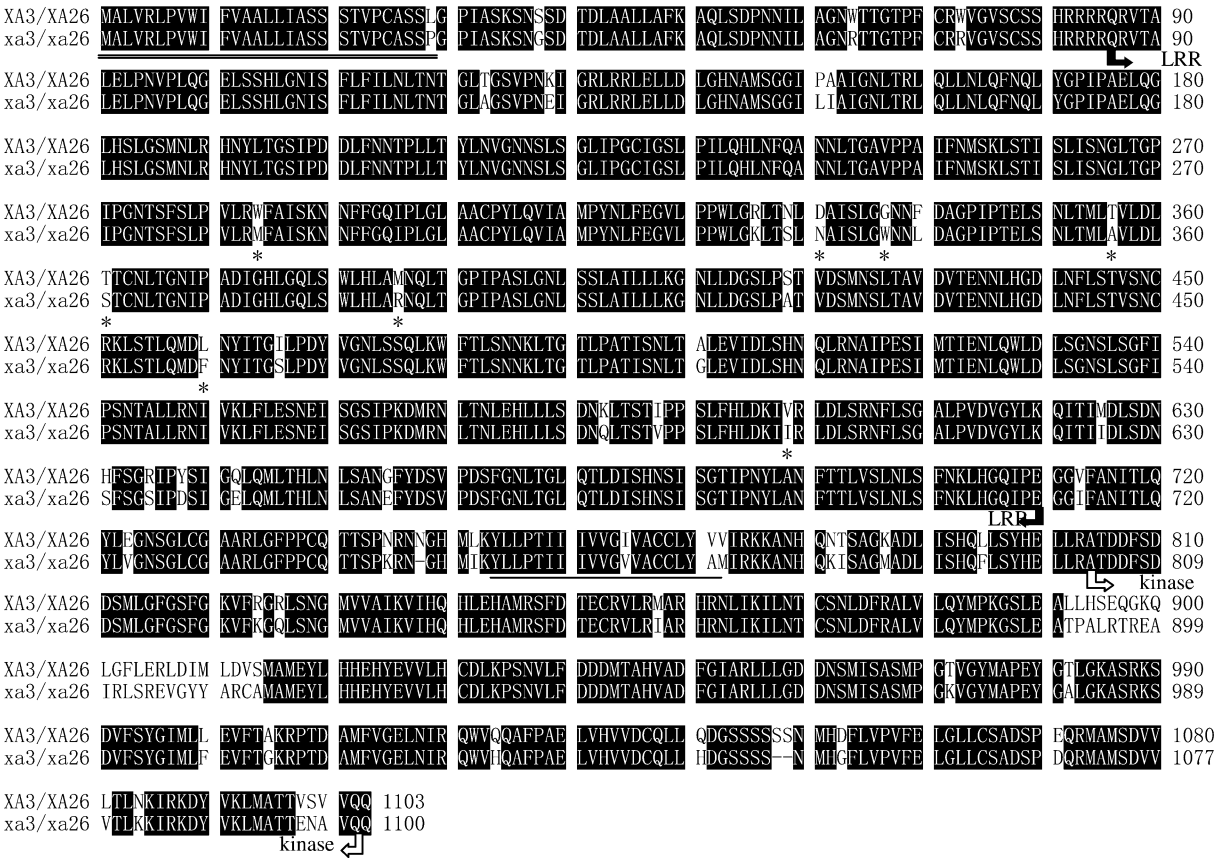

Fig. 6 Comparison of the predicted amino acid sequences of XA3/XA26 from rice lines IRBB3 and Minghui 63 and xa3/xa26 from rice line IR24. The identical amino acid residues of the two proteins are highlighted by *solid black shade*. The characterized structures of XA3/XA26 are indicated according to previous publication (Sun et al. 2004). The signal peptide sequence is *double underlined*. The region of LRR domain is indicated by a pair of *solid arrows*. The transmembrane region *is underlined*. The region of kinase domain is indicated by a pair of *hollow arrows*. The divergent solvent-exposed amino acid residues are marked with *asterisks*

resistance spectrum, *Xa26* performed variably at different growth stages in different genetic backgrounds (Yang et al. 2003; Sun et al. 2004). It conferred full resistance at both seedling and adult stages in the backgrounds of Minghui 63 and Mudanjiang 8, but it only conferred partial resistance at seedling stage and full resistance at adult stage in IRBB3.

Most of the characterized *R* genes and their susceptible alleles encode different proteins that determine the initiation of resistance or pathogenesis in host plants upon pathogen infection (Martin et al. 2003). Even a single amino acid difference can distinguish an *R* gene and its susceptible allele (Bryan et al. 2000). The exceptions are rice bacterial blight resistance gene *Xa27* and *xa13*; resistant and susceptible alleles of the two genes encode identical proteins (Gu et al. 2005; Chu et al. 2006). Induced expression of resistant allele but not susceptible allele upon infection is the basis of *Xa27*-mediated resistance, while promoter mutation resulting in down-regulation of expression during host-pathogen interaction is the basis of *xa13*-mediated resistance. *Xa3/Xa26* in IRBB3 and Minghui 63 and *xa3/xa26* in IR24 are constitutively expressed, which is not influenced by the inoculation of *Xoo* (Sun et al. 2004). IRBB3 and IR24 are near-isogenic lines that presumably have genetic difference only at *Xa3/Xa26* locus. Thus, the different reactions of IRBB3 and IR24 to *Xoo* could attribute to the sequence divergence between XA3/XA26 and *xa3/xa26* proteins. The solvent-exposed amino acid residues of LRR domain in R proteins are suggested to form a solvent-exposed surface that is involved in ligand binding (Jones and Jones 1997). It has been reported that the hypervariability of solvent-exposed amino acids correlates with differential pathogen recognition (Parniske et al. 1997; Thomas et al. 1997; Botella et al. 1998; Dixon et al. 1998; McDowell et al. 1998; Meyers et al. 1998; Ellis et al. 1999). Thus, the sequence difference occurred in the LRR domains, specifically at the solvent-exposed amino acid residues, may be the major cause that

differentiates the resistant and susceptible proteins. Large numbers of divergent amino acid sites were also detected in the kinase domains between *XA3/XA26* and *xa3/xa26* proteins. Since *xa3/xa26* is constitutively expressed in rice line IR24 (Sun et al. 2004), further study is needed to examine whether the *xa3/xa26* gene has other function in rice.

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