A paralog of the MtN3/saliva family recessively confers race-specific resistance to Xanthomonas oryzae in rice

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ABSTRACT

Approximately one third of the identified 34 rice major disease resistance (R) genes conferring race-specific resistance to different strains of Xanthomonas oryzae pv. oryzae (Xoo), which causes rice bacterial blight disease, are recessive genes. However, only two of the recessive resistance genes have been characterized thus far. Here we report the characterization of another recessive resistance gene, xa25, for Xoo resistance. The xa25, localized in the centromeric region of chromosome 12, mediates race-specific resistance to Xoo strain PXO339 at both seedling and adult stages by inhibiting Xoo growth. It encodes a protein of the MtN3/saliva family, which is prevalent in eukaryotes, including mammals. Transformation of the dominant Xa25 into a resistant rice line carrying the recessive xa25 abolished its resistance to PXO339. The encoding proteins of recessive xa25 and its dominant allele Xa25 have eight amino acid differences. The expression of dominant Xa25 but not recessive xa25 was rapidly induced by PXO339 but not other Xoo strain infections. The nature of xa25-encoding protein and its expression pattern in comparison with its susceptible allele in rice–Xoo interaction indicate that the mechanism of xa25-mediated resistance appears to be different from that conferred by most of the characterized R proteins.

Key-words: Bacterial blight; dominance reversal; Oryza sativa; resistance gene; susceptibility gene; Xoo.

INTRODUCTION

Bacterial blight, caused by Xanthomonas oryzae pv. oryzae (Xoo), is one of the most destructive diseases of rice worldwide. Use of major disease resistance (R) genes, which mediates race-specific resistance, has been an effective and economical strategy to control this disease. More than 30 R genes conferring host resistance against various strains of Xoo have been identified (Zhang 2007; Cheema et al. 2008; Ruan et al. 2008; Korinsak et al. 2009; Wang et al. 2009; Guo, Zhang & Lin 2010; Miao et al. 2010; Ram et al. 2010). Although only six of them (Xa1, Xa3/Xa26, xa5, xa13, Xa21 and Xa27) have been isolated (Xiang et al. 2006; Chu & Wang 2007), the R genes against Xoo are distinctive from R genes against other pathogens in the following aspects.

First, approximately one third [xa5, xa8, xa13, xa15, xa19, xa20, xa24, xa26, xa28, xa31(t), and xa33(t)] of naturally occurring R genes against Xoo are recessive in nature (Zhang 2007; Ruan et al. 2008; Korinsak et al. 2009). However, most of the reported R genes in other crop–pathogen systems, including rice R genes against Magnaporthe grisea, are dominant in nature (Yang et al. 2008; Moffett 2009; Liu et al. 2010).

Second, although most of the characterized R genes, including rice R genes against M. grisea, encode nucleotide-binding (NB)-leucine-rich repeat (LRR)-type proteins, the R genes against Xoo appear to encode various types of proteins. The dominant Xa21 and Xa3/Xa26 encode LRR receptor kinase-type proteins (Song et al. 1995; Sun et al. 2004; Xiang et al. 2006). The dominant Xa1 encodes an NB-LRR protein (Yoshimura et al. 1998). The recessive xa5 encodes a gamma subunit of transcription factor IIA (Iyer & McCouch 2004; Ji et al. 2006). The dominant Xa27 encodes an unknown apoplastic protein (Wu et al. 2008a). The recessive xa13 encodes a plasma membrane protein of the MtN3/saliva family (Chu et al. 2006a,b).

Finally, R gene-mediated Xoo resistance appears to function in various mechanisms. It is generally accepted that R proteins, such as the NB-LRR type, function as immune receptors to initiate defence signalling by directly or indirectly recognizing the corresponding pathogen effectors in host–pathogen interactions (Caplan, Padmanabhan & Dinesh-Kumar 2008; Collier & Moffett 2009; Padmanabhan, Cournoyer & Dinesh-Kumar 2009). However, xa5- and xa13-mediated disease resistance is different from the resistance conferred by NB-LRR-type proteins. The dominant allele (Xa13; also named as Os8N3 or OsSWEET11) of recessive R gene xa13 is a susceptible gene (Yang, Sugio & White 2006; Yuan et al. 2009; Chen et al. 2010b). The Xoo strain that is incompatible to recessive xa13 is sensitive to copper, an essential micronutrient of plants and an important element for a number of pesticides in agriculture (Yuan et al. 2010). Xoo overcomes rice defence by activating dominant Xa13 that the encoding protein incorporates with another two proteins to remove copper from the xylem vessels, where Xoo multiplies and spreads to cause disease (Yuan et al. 2010). The recessive xa13 is a mutant of
Xoo-induced dominant Xa13 (Chu et al. 2006b). The expression of recessive xa13 is not induced on Xoo infection caused by promoter mutation is the key factor for xa13-mediated resistance (Yuan et al. 2009, 2011). The encoding protein of recessive xa5 is involved in Xoo transcription activator-like (TAL) effector-regulated host gene expression (Gu et al. 2009). Furthermore, Xa21 functions both as an R protein and as a pattern recognition receptor (PRR) by recognizing an evolutionarily conserved pathogen-associated molecular pattern (Lee et al. 2009). PRRs regulate basal resistance (Zipfel 2008; Nicaise, Roux & Zipfel 2009). Endocytosis of Xa21 from the plasma membrane may be associated with initiating resistance during Xoo infection (Chen et al. 2010a).

These listed differences between R genes against Xoo and R genes against other pathogens suggest that the rice–Xoo system may be a unique model for identifying novel molecular mechanisms of host–pathogen interaction. Characterization of more R genes against Xoo will facilitate evaluation of this inference. In a previous study, we identified and mapped a new R gene against Xoo in rice. This gene localized in rice chromosome 12 and was tentatively named as Xa25(t) (Chen, Wang & Zhang 2002). In the present study, we demonstrate that this gene is recessively regulated. Thus, it is renamed as xa25. The recessive xa25 mediates race-specific resistance against Xoo.

**MATERIALS AND METHODS**

**Mapping population**

A segregation population consisting of 5816 F3 plants was used for mapping the xa25 gene. This population was generated from 60 F2 plants developed from the cross between a susceptible rice variety Zhenshan 97 (Oryza sativa ssp. indica) and a resistant rice variety Minghui 63 (O. sativa ssp. indica). Minghui 63 carried R genes xa25 (named Xa25(t) in reference) and Xa3/Xa26 against Xoo (Chen et al. 2002; Sun et al. 2004; Xiang et al. 2006). The 60 F2 plants did not carry Xa3/Xa26 based on PCR amplification using gene-specific primers (Supporting Information Table S1) and were heterozygous at markers RM179 and RM28172, which harboured xa25/Xa25(t) at chromosome 12 (Chen et al. 2002). For confirmation of fine mapping results, 20 progenies (F3) of each of 12 recombinant F3 plants were further examined for their responses to Xoo infection. Another F2 plant, S6 that did not carry Xa3/Xa26 based on PCR amplification using gene-specific primers (Supporting Information Table S1) and homozygous as Minghui 63 at markers RM179 and RM28172, was identified. S6 was resistance to Xoo strain PXO339 as Minghui 63. The next generation of S6, S6-1, was used to represent Minghui 63 at xa25/Xa25(t) locus to cross with susceptible Zhenshan 97 for further analysis.

**Genetic analysis**

A sample of 255 random F3 plants from the mapping population was used for mapping the target gene. A molecular linkage map was constructed using the computer program MAPMAKER/EXP 3.0 (Lincoln, Daly & Lander 1992). The maximum-likelihood map order for markers and the chromosomal location of the target gene on the molecular linkage map were determined with a logarithm of odds threshold of 3.0. The genetic distance (centimorgans (cM)) between markers for fine mapping of the target gene using 795 highly resistant F3 plants (average lesion length < 2 cm) from the population was calculated using equation \( (N_1 + N_2)/N \times 100 \) (Wu et al. 2008b), in which \( N \) is the total number of the highly resistant plants surveyed, \( N_1 \) is the number of plants homozygous for the marker genotype of resistance Minghui 63, and \( N_2 \) is the number of plants heterozygous for the two parents.

**Pathogen inoculation**

To evaluate bacterial blight disease, plants were inoculated with Philippine Xoo strains, PXO61 (race 1), PXO99 (race 6), PXO339 (race 9) or PXO341 (race 10) at the seedling (four- to six-leaf) or adult [booting (panicle development)] stage by the leaf-clipping method (Chen et al. 2002). Because the parents of the mapping population, Minghui 63 and Zhenshan 97, had different lengths of life cycle and the F3 individuals in the population could not grow to booting stage at the same time, plants in the population were inoculated with Xoo at four- to six-leaf stage. Disease was scored by measuring the lesion length (cm) or the percent lesion area (lesion length/leaf length) at 2 weeks after inoculation. For analysing gene expression in response to Xoo infection, the 2 to 3 cm leaf fragments next to bacterial infection sites were used for RNA isolation.

The growth rate of Xoo in rice leaves was determined from three leaves at each time point by counting colony-forming units (cfu; Sun et al. 2004). Leaves were sterilized using 75% ethanol, ground separately, suspended in sterilized water and plated on potato agar media.

**Plasmid construction and rice transformation**

To transfer the dominant Xa25 gene, a 5.2 kb DNA fragment including 1.5 kb upstream of transcription start site of Xa25 and 540 bp downstream of the termination site of Xa25, was amplified using LA-Taq polymerase (Takara Biotechnology, Dalian, China) and primers Xa25F3/R3 (Supporting Information Table S1) from rice variety Zhenshan 97. After verification by sequencing, the DNA fragment was cloned into transformation vector pCAMBIA1301 (CAMBIA; http://www.cambia.org.au). Agrobacterium-mediated transformation was performed using calli derived from mature embryos of rice varieties Minghui 63 and Zhonghua 11 (O. sativa ssp. japonica) according to the procedures reported previously (Lin & Zhang 2005). Positive transgenic plants were verified by PCR amplification of marker gene \( \beta \)-glucuronidase (\( GUS \)) using gene-specific primers (Supporting Information Table S1). In the background of Minghui 63, the presence of transgene was verified by using a cleaved amplified...
polymorphic sequence (CAPS) marker generated using the PCR primer pair MZCF5/MZCR5 (Supporting Information Table S1); the polymorphic PCR fragments were determined by electrophoresis of HindII-digested PCR products in 3% agarose gel. In the background of Zhonghua 11, the presence of transgene was determined by an insertion/deletion (Indel) marker generated using the PCR primer pair ZZF1/ZZR1 (Supporting Information Table S1); the polymorphic PCR fragments were determined by electrophoresis in 3% agarose gel.

**Gene expression analysis**

The semi-quantitative RT-PCR was conducted as previously described (Wen, Chu & Wang 2003). In brief, an aliquot (5 μg) of total RNA was treated with DNase I to remove contaminating DNA and then used for RT-PCR. PCR primers are listed in Supporting Information Table S1. The sizes of RT-PCR products for recessive xa25, dominant Xa25 and actin genes were 764, 759 and 121 nt, respectively.

**Statistical analyses**

The significant differences between the samples of transgenic and wild-type plants were analysed by the pairwise t-test installed in the Microsoft Office Excel program. The significance between the observed and expected segregation patterns of the resistant and susceptible plants in the mapping population was analysed by the \( \chi^2 \) test.

**RESULTS**

**A recessive gene against Xoo localizes on the centromere region of chromosome 12**

A previous study revealed that an R gene Xa25(t) in rice variety Minghui 63 dominantly mediates race-specific resistance to Xoo strain PXO339 at the adult stage (Chen et al. 2002). To isolate Xa25(t), 255 random F3 plants from the mapping population were used to study their responses to PXO339 at the seedling stage. The distribution of the lesion length caused by PXO339 infection in the 255 individuals was bimodal (Fig. 1). With a lesion length of 3.3 cm used as the dividing point, the number of resistant and susceptible individuals was 55 (lesion length \( \leq 3.3 \) cm) and 200 (lesion length \( > 3.3 \) cm), respectively, which fits the expected 1:3 ratio \( (\chi^2 = 1.698, P > 0.05) \). This result indicates that the resistance of Minghui 63 to PXO339 was controlled by a single recessive gene at the seedling stage.

The dominant Xa25(t) was mapped to the centromeric region flanked by molecular markers R887 and G1314 on rice chromosome 12 (Chen et al. 2002). Four molecular markers, which were putatively flanked by R887 and G1314 (G1314 sequence unknown), were used to study whether the previously identified dominant Xa25(t) and the presently identified recessive \( R \) gene were the same gene. The four markers included three simple sequence repeat (SSR) markers, RM179, RM511 and RM28172 (http://www.Gramene.org; International Rice Genome Sequencing Project 2005), and one Indel marker MZ2 (Supporting Information Table S1) that was designed according to the draft genomic sequences of the parents of the mapping population (Xie et al. 2010). Among the markers, RM179 was approximately 2536 kb from R887 and RM28172 was putatively linked to G1314 according to rice genome sequence (http://www.Gramene.org). The four markers were used to screen the sample containing the 255 random individuals from the mapping population (Fig. 1). With the lesion length of the 255 plants as the criterion, the recessive R gene was mapped to the centromere region on chromosome 12 (Fig. 2a) as the dominant Xa25(t) (Chen et al. 2002). Because the recessive R gene and dominant Xa25(t) appear to be the same gene, we designated this recessive gene as xa25. The recessive xa25 locus cosegregated with marker RM511 was 1.2 cM from marker MZ2 on one side and 0.4 cM from marker RM28172 on the other side (Fig. 2a).

To further fine map the recessive xa25, 795 highly resistant individuals from the mapping population were evaluated using markers MZ2 and RM28172, which flanked xa25 (Fig. 2a). Six recombinants that showed recombination events between MZ2 and xa25 were identified from one side of xa25 (Fig. 2b, Supporting Information Table S2). Another six recombinants were identified using RM28172 from the other side of xa25. According to the draft genomic sequences of Minghui 63 and Zhenshan 97 (Xie et al. 2010), two other Indel markers, MZ4 and MZ7, which were located between RM511 and RM28172, were designed (Supporting Information Table S1). MZ4, RM511 and MZ7 were used to examine the 12 recombinants detected by MZ2 and RM28172. One recombinant that was detected by RM28172 was also detected by MZ7 (Fig. 2b, Supporting Information Table S2). No recombinant event was detected by MZ4 and RM511 in the 12 plants identified by MZ2 and RM28172 (Supporting Information Table S2).
susceptible Zhenshan 97 was respectively transferred into Os12g29220 allele driven by its native promoter from the recessive xa25 Minghui 63. The resistant coding sequence as the Os12g29220 included in the DNA fragment flanked by MZ2 and MZ7. (Fig. 2b). These results suggest that the recessive MZ2 on one side and 0.06 cM from MZ7 on the other side cosegregated with RM511 and MZ4 was 0.38 cM from which were resistant to Xoo strain PXO339 as the recombinant F3 plants. Thus, the fine location of the recessive MZ2 and MZ7 was 1233 kb.

The reliability of all the 12 recombinants, which defined the fine location of the recessive xa25, was further examined by progeny test. Twenty progenies (F2 plants) from each of the F1 recombinants were inoculated with Xoo strain PXO339 at seedling stage. All the F2 plants were resistant to PXO339 as the recombinant F1 plants. Thus, the xa25 locus cosegregated with RM511 and MZ4 was 0.38 cM from MZ2 on one side and 0.06 cM from MZ7 on the other side (Fig. 2b). These results suggest that the recessive xa25 is included in the DNA fragment flanked by MZ2 and MZ7.

Recessive xa25 belongs to the MtN3/saliva family

The physical distance between MZ2 and MZ7 was 1233 kb (http://www.gramene.org), which contained 181 genes, according to Rice Genome Annotation Project (RGAP; http://rice.plantbiology.msu.edu/). One (RGAP locus number: Os12g29220) of the 181 genes belonged to the MtN3/saliva family as the recessive R gene xa13 against Xoo (Chu et al. 2006a). Sequence comparison of the coding regions of Os12g29220 alleles in resistant Minghui 63 and susceptible Zhenshan 97 showed 3% diversity, which resulted in amino acid sequence difference. The Os12g29220 alleles in rice varieties Zhonghua 11, Mudanjiang 8 (O. sativa ssp. japonica) and Nipponbare (O. sativa ssp. japonica), which were resistant to Xoo strain PXO339 (lesion areas 4.3 ± 1.0, 5.0 ± 1.7 and 3.1 ± 1.8%, respectively), had an identical coding sequence as the Os12g29220 allele in Minghui 63.

To ascertain whether Os12g29220 was the candidate of the recessive xa25, a DNA fragment harbouring the Os12g29220 allele driven by its native promoter from the susceptible Zhenshan 97 was respectively transferred into resistant Minghui 63 and Zhonghua 11. In total, 20 and 19 independent transformants with the genetic backgrounds of Minghui 63 (D175OMH) and Zhonghua 11 (D175OZH) were generated, respectively. All the T0 plants in Minghui 63 and Zhonghua 11 carrying the transgene Os12g29220 from Zhenshan 97 were susceptible to Xoo strain PXO339. The lesion areas of the 15 positive transgenic plants in Minghui 63 background ranged from 30.6% ± 7.4 to 50.6% ± 3.9% compared with 6.5% ± 1.1% for wild-type Minghui 63 and 49.9% ± 7.6% for susceptible control Zhenshan 97 (Supporting Information Table S3). The growth rate of PXO339 in transgenic plants carrying Os12g29220 from Zhenshan 97 was 5.4- to 35.1-fold higher than that in wild-type Minghui 63 in 4 to 12 d after infection at seedling stage (Fig. 3).

Transgenic plants carrying Os12g29220 from Zhenshan 97 also showed increased susceptibility to PXO339 in Zhonghua 11 background (Fig. 4a). The lesion areas of positive transgenic plants in Zhonghua 11 background ranged from 19.7% ± 2.9 to 31.3% ± 7.4% compared with 4.3% ± 1.0% for wild-type Zhonghua 11 (Supporting Information Table S3).

To confirm that the increased susceptibility of the positive transgenic plants was because of transgene, T1 families derived from each three of the susceptible T0 plants with Minghui 63 and Zhonghua 11 backgrounds were investigated individually for susceptibility and the existence of transgene. The increased susceptibility of the T1 plants cosegregated with the existence of transgene in all six T1 families (Supporting Information Table S3). These results suggest that the Os12g29220 allele in resistant Minghui 63 is the recessive xa25. The xa25 confers resistance by inhibiting Xoo growth. Because the resistance of Minghui 63 to Xoo strain PXO339 was compromised by expression of dominant xa25, the previously named R gene Xa25(t) that dominantly conferred Minghui 63 resistance to PXO339 (Chen et al. 2002) should be the same gene as the recessive xa25.

Recessive \( x_{a25} \) mediates race-specific resistance

Transgenic plants carrying dominant \( Xa25 \) was analysed for its responses to other \( Xoo \) strains at both seedling and adult stages. In seedling stage, the wild-type Minghui 63 carrying dominant \( Xa3/Xa26 \) and recessive \( xa25 \) was resistant to \( PXO339 \) and susceptible to \( PXO61, PXO341 \) and \( PXO99 \). The transgenic plants showed significantly increased susceptibility \((P < 0.01)\) to \( PXO339 \) as compared with the wild-type and the susceptible control plants (Zhenshan 97) and had the same level of susceptibility to
PXO61, PXO341 and PXO99 (Fig. 4b). In the adult stage, the wild-type Minghui 63 was resistant to PXO339, moderately resistant to PXO61 and PXO341, and susceptible to PXO99 (Fig. 4b). Similar to the seedling stage, the transgenic plants only showed significantly increased susceptibility (P < 0.01) to PXO339 but not Xoo strains PXO61, PXO341 and PXO99 as compared with wild-type plants at adult stage (Fig. 4b). These results suggest that the recessive xa25 confers race-specific resistance to PXO339 at both seedling and adult stages.

**Dominant Xa25 but not recessive xa25 is specifically induced by PXO339**

To study whether pathogen infection influenced the expression of recessive xa25 and dominant Xa25, resistant Minghui 63 and susceptible Zhenshan 97 were inoculated with different Xoo strains (Fig. 5). Xoo strain PXO339 induced the expression of dominant Xa25 in Zhenshan 97 but not recessive xa25 in Minghui 63 in seedling stage (Fig. 5a). Other Xoo strains (PXO61, PXO99 and PXO341) did not influence the expression of xa25 and Xa25 nor did PXO339 induce the recessive xa25 in resistant Zhonghua 11, Mudanjiang 8 and Nipponbare (Fig. 5b). PXO339 also induced dominant Xa25 but not recessive xa25 in adult stage (Fig. 5c). The consistency of PXO339-regulated race-specific susceptibility and PXO339-induced Xa25 expression suggests that the activation of dominant Xa25 may be associated with susceptibility.

To ascertain whether the differential expression of dominant Xa25 and recessive xa25 was associated with their promoter difference, the promoter regions (approximately 1.11 kb upstream of the transcription initiation site) of xa25/Xa25 were compared. The Xa25 promoter from susceptible rice variety Zhenshan 97 was different from xa25 promoters from resistant rice varieties Minghui 63, Zhonghua 11, Nipponbare and Mudanjiang 8 because of nucleotide substitutions, insertions and deletions (Supporting Information Fig. S1). The xa25 promoters from Nipponbare and Mudanjiang 8 had identical sequence but different from the xa25 promoters from Minghui 63 and Zhonghua 11. In addition, the xa25 promoters from Minghui 63 and Zhonghua 11 are also different from each other. However, seven polymorphic sites, −1117 (T/C), −1075 (T/C), −663 (deletion/T), −248 (A/G), −56 (C/G), −40 (G/T) and −28 (A/deletion) according to the

![Diagram](image-url)  
**Figure 5.** The influence of Xoo infection on xa25/Xa25 expression analysed by RT-PCR. ck, before pathogen inoculation; 1, 2 and 3 d after inoculation of Xoo. The number on the right of each gel picture indicates the cycles of PCR reaction. (a) Only infection of Xoo strain PXO339 induced the expression of dominant Xa25 in susceptible rice variety Zhenshan 97 at seedling stage. (b) PXO339 infection did not influence the expression of recessive xa25 in resistant rice varieties Zhonghua 11, Mudanjiang 8 and Nipponbare at seedling stage. (c) PXO339 induced the expression of dominant Xa25 in Zhenshan 97 at booting stage.

nucleotide position in Minghui 63, between the promoters of recessive \( xa25 \) from the four resistant rice varieties and dominant \( Xa25 \) from susceptible Zhenshan 97, were identified. This result suggests that the differential expression of dominant \( Xa25 \) and recessive \( xa25 \) in response to PXO339 infection may be associated with their promoter difference.

**Recessive \( xa25 \) and dominant \( Xa25 \) encode different proteins**

Sequence comparison of the genomic and cDNA (GenBank accession number: AK242853; from rice variety Nipponbamer) sequences of recessive \( xa25 \) from resistant Minghui 63, Zhonghua 11, Mudanjiang 8 and Nipponbamer, and dominant \( Xa25 \) from susceptible Zhenshan 97, showed that the coding regions of the recessive and dominant alleles were interrupted by five introns (Supporting Information Fig. S2). The recessive \( xa25 \) alleles in Minghui 63, Zhonghua 11, Mudanjiang 8 and Nipponbamer putatively encode identical protein consisting of 296 amino acids. The dominant \( Xa25 \) putatively encodes proteins consisting of 293 amino acids (Supporting Information Fig. S3). In addition to the size difference, the two proteins have five-residue substitutions (Supporting Information Fig. S3). These results suggest that the different functions of recessive \( xa25 \) and dominant \( Xa25 \) may also be associated with the differences in their encoding proteins.

**Developmental stage influences \( xa25 \)-mediated resistance**

Chen et al. (2002) reported that \( xa25/Xa25(t) \) dominantly regulated resistance to \( Xoo \) strain PXO339 in a mapping population at adult stage, but the present results reveal that \( xa25 \) recessively regulate resistance to PXO339 in a similar mapping population at seedling stage. To analyse the inconsistency, different plants were grown by staggered planting, so that all the plants with different genetic background and at seedling (four- to six-leaf) and adult (booting) stages could be inoculated with PXO339 at the same time. Minghui 63 carried \( R \) genes \( Xa3/Xa26 \) and \( xa25 \) for \( Xoo \) resistance. Rice \( S6-1 \) was a \( F_1 \) line developed from the cross between susceptible Zhenshan 97 and resistant Minghui 63; \( S6-1 \) did not carry \( Xa3/Xa26 \) but it was homozygous at \( xa25 \) locus as Minghui 63. \( F_1 \) was the hybrid from the cross between Zhenshan 97 and Minghui 63, and \( F_1-1 \) was the hybrid from the cross between Zhenshan 97 and \( S6-1 \). Although \( xa25 \) functioned as a recessive gene in transgenic plants at both seedling and adult stages (Fig. 4b), it functioned as a recessive gene in seedling stage but like a dominant or partial dominant gene at adult stage in natural condition based on the performance of the \( F_1 \) and \( F_1-1 \) plants to PXO339 infection (Fig. 6a). The lesion areas of \( F_1 \) and \( F_1-1 \) plants at adult stage were only one of fifth of the lesion areas at seedling stage. Minghui 63 and \( S6-1 \) were slightly more resistance to PXO339 at adult stage than at seedling stage. Zhenshan 97 was markedly less susceptible to PXO339 at adult stage than at seedling stage. These results suggest that \( xa25 \)-mediated resistance is influenced by developmental stage.

The expression of recessive \( xa25 \) and dominant \( Xa25 \) in the same staggered planted plants were analysed by RT-PCR (Fig. 6b). The expression of dominant \( Xa25 \) in susceptible Zhenshan 97 was induced after PXO339 infection at both seedling and adult stages. PXO339 did not induce the recessive \( xa25 \) in resistant Minghui 63 and \( S6-1 \). Interestingly, the expression \( xa25/Xa25(t) \) in \( F_1 \) and \( F_1-1 \) plants was induced only at seedling stage but not at adult stage after PXO339 infection. These results suggest that development-regulated \( xa25 \)-mediated resistance may be influenced by the expression level of its dominant allele \( Xa25 \) when the \( xa25/Xa25 \) locus is heterozygous.

**DISCUSSION**

The recessive \( xa25 \) is the same as \( Xa25(t) \)

The results of fine genetic mapping and functional complementation analysis suggest that \( xa25 \) is a recessive \( R \) gene at both seedling (Figs 3 & 4a,b, Supporting Information Table S3) and adult (Fig. 4b) stages. The recessive \( xa25 \) is the same gene as previously named \( Xa25(t) \) (Chen et al. 2002). However, genetic analyses showed that \( xa25 \) was recessively regulated at seedling stage (Fig. 1) but dominantly regulated at adult stage [thus named \( Xa25(t) \) in Chen et al. 2002]. The inconsistent results of the genetic analyses may have the following explanations. Firstly, the recessive \( xa25 \) may be an \( R \) gene with the characteristics of dominance reversal. Some rice \( R \) genes against \( Xoo \) function recessively at an early developmental stage and dominantly at a late developmental stage (Sidhu & Khush 1978; Zhao, Zhang & Xie 1986). The characteristics of rice plants showed that when they were heterozygous for the target \( R \) gene, they were susceptible at an early development stage but resistant at a late developmental stage (Sidhu & Khush 1978; Zhao et al. 1986). However, the molecular mechanism controlling this reversal of dominance is unknown. Rice plants carrying \( xa25/Xa25(t) \) have the same characteristic as the rice varieties carrying \( R \) genes with the nature of dominance reversal reported previously (Sidhu & Khush 1978; Zhao et al. 1986). The \( F_1 \) plants developed from the cross between susceptible Zhenshan 97 (carrying dominant susceptible \( Xa25 \)) and resistant Minghui 63 (carrying recessive resistant \( xa25 \)) were susceptible (lesion length 11.2 cm) to \( Xoo \) strain PXO339 compared with Zhenshan 97 (lesion length 12.9 cm) and Minghui 63 (lesion length 3.5 cm) at seedling stage; the same \( F_1 \) plants became resistant (lesion length 3.5 cm) to PXO339 compared with Zhenshan 97 (lesion length 11 cm) and Minghui 63 (lesion length 1 cm) at the adult stage (Chen 2001). The present results further confirm the characteristic of dominance reversal of \( xa25 \)-carrying plant (Fig. 6a). In addition, the present results
suggest that this dominance reversal appears to be associated with suppressing *Xoo*-induced expression of dominant *Xa25* (Fig. 6b), although further study is required to examine this inference.

Secondly, development-associated minor resistance quantitative trait loci (QTLs) may influence the function of the recessive *xa25*. This hypothesis is supported by the characteristic of another rice *R* gene *Xa3/Xa26* for *Xoo* resistance, although further study is required to examine this hypothesis. *Xa3/Xa26* has a dosage effect that is regulated by rice development; this dosage effect is associated with enhanced expression of defence-responsive genes *OsWRKY13* and *NH1* (Cao et al. 2007). *OsWRKY13* and *NH1* function as minor resistance QTLs in rice–pathogen interactions (Hu et al. 2008; Kou et al. 2010).

**MtN3/saliva-type proteins may have different biochemical functions**

The recessive *xa25* belongs to the MtN3/saliva gene family. The only known structure of *xa25/Xa25* proteins are MtN3/saliva domain. This domain was originally identified in root
nodulin-related proteins of legume (Gamas et al. 1996) and late in the saliva protein of Drosophila (Artero et al. 1998). MtN3/saliva family proteins are prevalent in eukaryotes including mammals (Guan et al. 2008), suggesting that they may have important roles in the physiological and developmental activities of eukaryotes, but their biochemical functions are largely unknown. Until recently, the biochemical functions of several MtN3/saliva-type proteins are characterized. Rice susceptible protein Xa13 interacts with rice copper transporter 1 (COPT1) and COPT5 to remove copper from xylem vessels in the rice–Xoo interaction. The removal of copper from xylem may be associated with transporting copper into cells, because only the coexpression of the three plasma membrane proteins could complement the phenotype of yeast mutant that lacked the functions of copper transporters for copper uptake (Yuan et al. 2010). The MtN3/saliva-type proteins are recently also named SWEETs; several SWEETs from Arabidopsis, rice, Caenorhabditis elegans and humans can mediate glucose transport in mammalian cells, yeast cells and oocytes (Chen et al. 2010b). The Xa13 (also named OsSWEET11) functions as a low-affinity glucose transporter in mammalian cells and oocytes (Chen et al. 2010b). In addition, the rat RGA protein that is distantly related to the MtN3/saliva-type proteins plays a role in trafficking an ion channel protein to plasma membrane (Barnhill et al. 2004; Stokes et al. 2005). These results suggest that MtN3/saliva-type proteins may have different biochemical functions.

The rice MtN3/saliva gene family consists of at least 23 paralogs according to Chen et al. (2010b) and rice genome annotation (http://rice.plantbiology.msu.edu/). The encoding proteins of xa25/Xa25 are most closely related to OsSWEET14 (also named Os11N3) based on the phylogenetic analysis (Fig. 7). The OsSWEET14/Os11N3 functions as a low-affinity transporter to mediate glucose efflux in mammalian cells and oocytes; it is suggested that this function of OsSWEET14/Os11N3 may be used by pathogens for nutritional gain (Chen et al. 2010b). Further study is required to elucidate whether xa25/Xa25 is also involved in sugar transporter in rice–Xoo interaction.

The models for the molecular mechanism of xa25-mediated resistance

The present results suggest two putative alternative models for the molecular mechanism of xa25-mediated resistance. One model is that the dominant Xa25 may be a race-specific susceptibility gene and the recessive xa25 is a Xoo-induced expressionional non-reaction mutant of the dominant Xa25. This inference is supported by the evidence that specific Xoo strain-induced transcriptional activation of dominant Xa25 was associated with race-specific susceptibility (Figs 4b & 5). This inference is also supported by the following indirect evidence. Two other paralogs (Xa13 and OsSWEET14/Os11N3) of rice MtN3/saliva family have been reported to function as Xoo race-specific susceptibility genes. Xoo-induced Xa13 expression is associated with rice susceptibility (Chu et al. 2006b; Yuan et al. 2009). The activation of Xa13 is executed by binding a specific Xoo TAL effector to the UPT box in Xa13 promoter (Römer et al. 2010; Yuan et al. 2011). The recessive resistance gene xa13 is a loss-susceptibility mutant of the dominant Xa13 (Yuan et al. 2009). The OsSWEET14/Os11N3-involved rice race-specific susceptibility to Xoo is also associated with specific TAL effector-induced activation of this paralog (Antony et al. 2010; Römer et al. 2010). In addition, a paralog of pepper MtN3/saliva family was induced by a TAL effector (Kay et al. 2009). A number of paralogs of Arabidopsis MtN3/saliva family are differentially induced by bacterial and fungal pathogens (Ferrari et al. 2007; Chen et al. 2010b). These results suggest that MtN3/saliva family genes may be widely involved in host–pathogen interactions.

An alternative model is that the resistance function of xa25 may be because of its encoding protein being different from that encoded by its dominant allele Xa25. Almost all the characterized R genes are distinguished from their susceptible alleles by encoding different proteins even with only one amino acid difference, such as rice xa5 against Xoo (Iyer & McCouch 2004) and rice Pi-ta against M. grisea that causes blast disease (Bryan et al. 2000).

In conclusion, the nature of xa25-encoding protein and its expression pattern in comparison with its susceptible allele in rice–Xoo interaction indicate that the mechanism of xa25-mediated resistance appears to be different from that conferred by most of the characterized R proteins


![Figure 7. Phylogenetic analysis of xa25/Xa25 proteins with other 22 paralogs in rice MtN3/saliva family. The tree was constructed by the neighbour-joining method. The numbers for interior branches indicate the bootstrap values (%) for 1000 replications. The scale is in units of number of amino acid substitutions per site.](image-url)
harbouring NB or LRR domains. Thus, xa25 can be used as another sample to study novel molecular mechanism of host–pathogen interactions. The present results provide further focus to elucidate the molecular mechanism of xa25-mediated resistance.

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REFERENCES


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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Figure S1. Alignment of the promoter regions of recessive xa25 from resistant rice varieties Minghui 63, Zhonghua 11, Nipponbare and Mudanjiang 8, and dominant Xa25 from susceptible rice variety Zhenshan 97. The nucleotide immediately upstream of the transcription initiation site is numbered as ‘-1’. The polymorphic sites in Zhenshan 97 compared with the four resistant rice varieties are marked in red.

Figure S2. The structure of recessive xa25 from rice variety Minghui 63. The coding region (black boxes) of xa25 is interrupted by five introns (thick lines). The positions of 5’- and 3’-untranslated regions (hatched boxes), translation start codon (ATG) and translation stop codon (TAG) are shown. The numbers indicate the base pairs of each substructure.
Figure S3. Sequence comparison of the xa25 and Xa25 proteins. The xa25 protein is encoded by recessive R gene xa25 in rice varieties Minghui 63, Zhonghua 11, Nipponbare and Mudanjiang 8. The Xa25 protein is encoded by dominant susceptible gene Xa25 in rice variety Zhenshan 97.

Table S1. Primers used for PCR amplification.

Table S2. Marker genotypes of 12 recombinant individuals from highly resistant individuals (n = 795) and their reactions to the Xoo strain PXO339.

Table S3. Response of transgenic plants carrying Os12g29220 (Xa25) allele from susceptible rice variety Zhenshan 97 to Xoo strain PXO339 at seedling stage.

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