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Functional analysis of *Xa3/Xa26* family members in rice resistance to *Xanthomonasoryzae* pv. *oryzae*

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Abstract Plant disease resistant (*R*) genes are frequently clustered in the genome. The diversity of members in a complex *R*-gene family may provide variation in resistance specificity. Rice *Xa3/Xa26*, conferring resistance to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) encodes a leucine-rich repeat (LRR) receptor kinase-type protein and belongs to a multigene family, consisting of *Xa3/Xa26*, *MRKa*, *MRKc* and *MRKd* in rice cultivar Minghui 63. *MRKa* and *MRKc* are intact genes, while *MRKd* is a pseudogene. Complementary analyses showed that *MRKa* and *MRKc* could not mediate resistance to *Xoo* when regulated by their native promoters, but *MRKa* not *MRKc* conferred partial resistance to *Xoo* when regulated by a strong constitutive promoter. Plants carrying truncated XA3/XA26, which lacked the kinase domain, were compromised in their resistance to *Xoo*. However, the kinase domain of MRKa could partially restore the function of the truncated XA3/XA26 in resistance. *MRKa* and *MRKc* showed similar expression pattern as *Xa3/Xa26*, which expressed only in the vascular systems of different tissues. The expressional characteristic of *MRKa* and *MRKc* perfectly fits the function of genes conferring resistance to *Xoo*, a vascular pathogen. These results suggest that although *MRKa* and *MRKc* cannot mediate

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bacterial blight resistance nowadays, they may be once effective genes for *Xoo* resistance. Their expressional characteristic and sequence similarity to *Xa3/Xa26* will provide templates for generating novel recognition specificity to face the evolution of *Xoo*. In addition, both LRR and kinase domains encoded by *Xa3/Xa26* and *MRKa* are the functional determinants and *MRKa*-mediated resistance is dosage-dependent.

Introduction

Plants face a diversity of pathogens, including bacteria, fungi, nematodes, oomycetes, and viruses, throughout their life circle. They respond to these biotic attackers by activating the encoding products of disease resistance (*R*) genes, which in turn trigger defense signal transduction cascades leading to rapid and race-specific disease resistance in host plants. More than 40 *R* genes have been isolated from dicotyledonous and monocotyledon plants (Martin et al. 2003). Most of the characterized *R* genes encoding proteins containing conserved nucleotide-binding site (NBS) domain and/or leucine-rich repeat (LRR) domain. *R* genes tend to be clustered in the genome. The diversity of members in a complex *R*-gene family may provide variation in resistance specificity. Rice *Xa21* gene conferring race-specific resistance to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), which causes bacterial blight disease, is one member of the *Xa21* multigene family (Song et al. 1995); another member of this family, the *Xa21D*, mediates partial resistance to *Xoo* (Wang et al. 1998). Rice *Pi2*, *Piz-t* and *Pi9* mediating race-specific resistance to *Magnaporthe grisea* are different members of the same gene family (Qu et al. 2006; Zhou et al. 2006). Tomato *R* genes *Cf4* and *Cf9* conferring race-specific resistance to *Cladosporum fulvum* belong to the

same family; at least two other members in the *Cf4/Cf9* family also mediate resistance to *Cladosporum fulvum* in a race-specific manner (Parniske et al. 1997).

Rice *Xa26* gene, conferring race-specific resistance to *Xoo*, encodes LRR receptor kinase-type protein (Sun et al. 2004). Among the large numbers of characterized *R* genes, only *Xa26* and *Xa21* encoding this type of proteins (Song et al. 1995), although the *Arabidopsis FLS2* gene functioning in race-nonspecific basal immunity also encodes a LRR receptor kinase and rice blast resistance gene *Pi-d2* encoding a B-lectin receptor kinase (Gomez-Gomez and Boller 2000; Chen et al. 2006). Our previous studies have shown that *Xa26* belongs to a multiple gene family with all the members clustered in tandem on rice chromosome 11 (Sun et al. 2004, 2006); this family consists of four members, *MRKa*, *Xa26* (*MRKb*), *MRKc* and *MRKd* in rice cultivar Minghui 63 (GenBank accession number DQ355952); *MRKa* and *MRKc* are predicted to be intact genes, whereas *MRKd* is predicted to be a pseudogene carrying one in-frame stop codon, two frameshift sites, and two inserts of 5,881 and 2,092 bp, respectively. The *Xa26* locus is tightly linked to another bacterial blight resistance gene *Xa4*; fine genetic mapping of *Xa4* suggests that it is likely a member of the *Xa26* family but is not allelic to *Xa26* (Sun et al. 2003; Yang et al. 2003). In addition, several other rice resistance genes, *Xa22(t)* for bacterial blight resistance and *Pi1*, *Pi18(t)*, and *Pi44(t)* for fugal blast resistance, are also mapped to the chromosomal region that corresponds to the location of *Xa26* family, which suggests that these noncharacterized rice resistance genes could be also the members of this family (Ahn et al. 1996; Lin et al. 1996; Yu et al. 1996; Chen et al. 1999). Evolutionary analysis showed that *Xa26* family had extensive paralog diversity as compared with the rice *R* gene *Xa21* family and tomato *R* gene *Cf-4/9* family, indicating that *Xa26* family may be an evolutionary old family and/or it has been subject to a higher rate of mutation (Parniske et al. 1997; Song et al. 1997; Sun et al. 2006). Thus analysis of the functions of *Xa26* family members in disease resistance will facilitate the identification of new *R* genes and full understanding of the function of *Xa26* family.

Our recent study has shown that *Xa26* is the same gene as *Xa3* (Xiang et al. 2006), which is known to be an important bacterial blight resistance gene in the *japonica* cultivar breeding in China (Xu et al. 2004). Thus we name this gene *Xa3/Xa26* to indicate the relationship between the two gene symbols. In this study we analyzed the function of another two members of *Xa3/Xa26* family, *MRKa* and *MRKc*, in rice resistance to *Xoo* by two types of methods, overexpression and domain swap that are commonly used strategies for studying the functions of *R* or *R*-like genes (Tang et al. 1999; Wulff et al. 2001; Hwang and Williamson 2003).

Materials and methods

Plasmid construction

To construct the transformation vectors containing target genes regulated by their native promoters, a 6.5-kb DNA fragment harboring *MRKa* and its native promoter and a 9.6-kb DNA fragment harboring *MRKc* and its native promoter were obtained by digestion of the bacterial artificial chromosome (BAC) clone 3H8 from rice cultivar Minghui 63 (*Oryza sativa* ssp. *japonica*) with restriction enzyme *Dra*I and *Bgl*II, respectively. Both fragments were individually ligated with vector pCAMBIA1301 (Sun et al. 2004).

To construct the overexpression vectors for *MRKa*, *MRKc*, *Xa3/Xa26LT* (a truncated *Xa3/Xa26*), and *Xa3/Xa26LT-MRKaK* (a chimeric gene between *Xa3/Xa26* and *MRKa*), the DNA fragments harboring the coding regions of target genes were obtained by PCR amplification using BAC clone 3H8 as template and gene-specific primers (Supplemental Table 1). Each DNA fragment was inserted into vector pU1301, which contained a maize ubiquitin gene promoter to regulate the expression of target gene (Qiu et al. 2007).

To construct an RNA interference (RNAi) vector of *Xa3/Xa26*, a 365-bp DNA fragment of *Xa3/Xa26* amplified using primers MKbDSF and MKbDSR from BAC clone 3H8 was inserted into pDS1301 vector (Chu et al. 2006). The MKbDSF contained *Spe*I and *Kpn*I restriction enzyme sites at the 5′-end, and MKbDSR contained *Sac*I and *Bam*HI restriction enzyme sites at the 5′-end (Supplemental Table 1). The sequences following the restriction enzyme sites of MKbDSF and MKbDSR were complementary to the sequence of *Xa3/Xa26*.

For studying the expression patterns of *Xa3/Xa26*, *MRKa* and *MRKc*, the promoters of the three genes were fused with reporter gene *GFP* (green fluorescent protein) and/or *GUS* (β-glucuronidase). To construct P_{Xa26}:*GFP* or P_{Xa26}:*GUS*, a DNA fragment, locating 1,160-bp upstream and 544-bp downstream of the translation start codon of *Xa3/Xa26* and harboring the *Xa3/Xa26* promoter, was obtained by digestion using restriction enzyme *Bam*HI and *Pst*I from rice cultivar Minghui 63. For preparing the constructs carrying P_{MRKa}:*GFP* and P_{MRKc}:*GFP*, DNA fragments locating 658 and 1,060-bp upstream and 32 and 71-bp downstream of the predicted transcription initiation sites of *MRKa* and *MRKc* and harboring the promoters of *MRKa* and *MRKc*, respectively, were obtained by PCR amplification using 3H8 as template and gene-specific primers (Supplemental Table 1). Each promoter was fused with *GFP* and/or *GUS* and cloned into the pCAMBIA1381 vector.

Plant transformation

All the constructs were transferred into *Agrobacterium tumefaciens* strain EHA105, which was kindly provided by the Center for the Application of Molecular Biology to International Agriculture, by electroporation. *Agrobacterium*-mediated transformation was performed using calli derived from mature embryos of rice cultivars Mudanjiang 8 (Sun et al. 2004, *O. sativa* ssp. *japonica*), Zhonghua 11 (Yuan et al. 2007, *O. sativa* ssp. *japonica*) or Minghui 63 by following the procedure reported by Lin and Zhang (2005).

Bacterial inoculation

Rice plants were inoculated with Chinese *Xoo* strain Z173 (group 4), Japanese strains T1, T2 or T3, or Philippine strains PXO61 (race 1), PXO79 (race 3), PXO71 (race 4), PXO112 (race 5), PXO99 (race 6), PXO145 (race 7), or PXO280 (race 8) at the booting stage by using leaf-clipping method (Sun et al. 2004). Mock-inoculated (control) plants were treated under the same condition except that bacterial inoculum was replaced with ddH$_2$O. The bacterial inoculum was prepared as described previously by Sun et al. (2004). Disease was scored by measuring the lesion length (cm) at 14 days after inoculation.

Reverse transcription-PCR

Reverse transcription (RT)-PCR was performed using gene-specific primers (Supplemental Table 1) as described by Wen et al. (2003). Quantitative PCR (qPCR) was conducted using the ABI 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA), according to the manufacturer's instruction. The expression level of actin was used to standardize the RNA sample for each RT-qPCR. The qPCR reaction was in a 25-μl volume containing 1 μl of diluted reverse transcription product, 12.5 μl of 2× SYBR Green PCR Master Mix (Applied Biosystems), and 0.32 μM of each primer. For each analysis, RT-qPCR assays were repeated at least twice with each repetition having three replicates; similar results were obtained in repeated experiments.

Results

### Overexpression of *MRKa* enhances bacterial resistance in rice

To determine whether the intact members of *Xa3/Xa26* family in Minghui 63 possess disease resistance function, the expression of *MRKa* and *MRKc* genes was first exam-

ined by RT-PCR analysis. The results showed that *MRKa* and *MRKc* genes expressed in leaf tissue (Fig. 1a). The two genes were then transferred into susceptible *japonica* rice cultivars Mudanjiang 8 and Zhonghua 11.

The *MRKa* gene with its native promoter (P_{MRKa}) was transferred to Mudanjiang 8 and Zhonghua 11. A total of 120 independent positive Mudanjing 8 transformants and nine independent positive Zhonghua 11 transformants were obtained. All transgenic plants carrying P_{MRKa}:*MRKa* were susceptible to *Xoo* strain PXO61 as the wild type (Fig. 1b). We then transferred *MRKa* driven by a strong constitutive promoter, maize ubiquitin gene promoter (P_{Ubi}), to Zhonghua 11. Eight independent positive transformants named MKaFZH were obtained. Five of the eight transgenic plants carrying P_{Ubi}:*MRKa* showed significantly enhanced ($P < 0.05$) resistance to PXO61 with the lesion length ranged from 4.2 ± 1.8 to 7.2 ± 1.8 cm as compared to 12.1 ± 1.4 cm measured for the control of wild-type Zhonghua 11 (Fig. 1b; Supplemental Table 2). The enhanced resistance was associated with overexpression of *MRKa* in the T_0 plants (Fig. 1c). T_1 families derived from three of the resistant T_0 plants, MKaFZH5, MKaFZH10 and MKaFZH13 carrying P_{Ubi}:*MRKa*, were further examined individually for resistance by inoculating with PXO61 and also for the presence of the transgene by PCR analysis at booting stage. It was shown that the enhanced resistance cosegregated with *MRKa* in all three T_1 families (Supplemental Table 2), indicating that the improved resistance was due to the existence of *MRKa*. The bacterial growth analysis demonstrated that the growth rate of PXO61 on resistant transgenic T_1 plants carrying P_{Ubi}:*MRKa* at the booting stage was 10.6-fold lower than that on wild type at 14 days after inoculation (Fig. 1d). These results suggest that overexpressing *MRKa* can enhance rice resistance to *Xoo*. However, the average lesion length of *MRKa*-overexpressing T_1 plants was approximately 7.4 cm longer than the plants carrying *Xa3/Xa26* in the same genetic background after PXO61 infection. Thus, overexpression of *MRKa* only mediated partial resistance to *Xoo*.

All the 20 independent transgenic plants carrying *MRKc* driven by its native promoter (P_{MRKc}) in Mudanjiang 8 background were susceptible to PXO61. We then overexpressed *MRKc* using P_{Ubi} in Mudanjiang 8. All the 16 independent transgenic plants carrying P_{Ubi}:*MRKc* and their T_1 generations were inoculated with different *Xoo* strains, including one Chinese strain (Z173), three Japanese strains (T1, T2, and T3), and seven Pillippine strains (PXO61, PXO79, PXO71, PXO112, PXO99, PXO145, and PXO280). All the transgenic plants were susceptible to the bacteria (data not shown). These results suggest that *MRKc* is not involved in the regulation of bacterial blight resistance.

Fig. 1 Expression of *Xa3/Xa26* family members and performance of transgenic plants in response to bacterial infection. Zhonghua 11 is a wild type. **a** *Xa3/Xa26* family members *MRKa* and *MRKc* expressed in the leaves of rice cultivar Minghui 63 analyzed by RT-PCR. **b** Leaves from T_0 transgenic plants carrying P_{Ubi}:*MRKa* or P_{MRKa}:*MRKa* at 14 days after inoculation with *Xoo* strain PXO61. **c** Enhanced resistance to PXO61 was associated overexpression of *MRKa* in T_0 transgenic plants analyzed by RT-qPCR. The PCR primers used for RT-qPCR could also detect a background signal in wild-type Zhonghua 11 and negative transgenic plants (1N and 9N) that did not carry *MRKa*. **d** Growth of PXO61 in leaves of T_1 plants at booting stage. The bacterial population was determined from three leaves at each date point by counting colony-forming units (*cfu*, Sun et al. 2004)
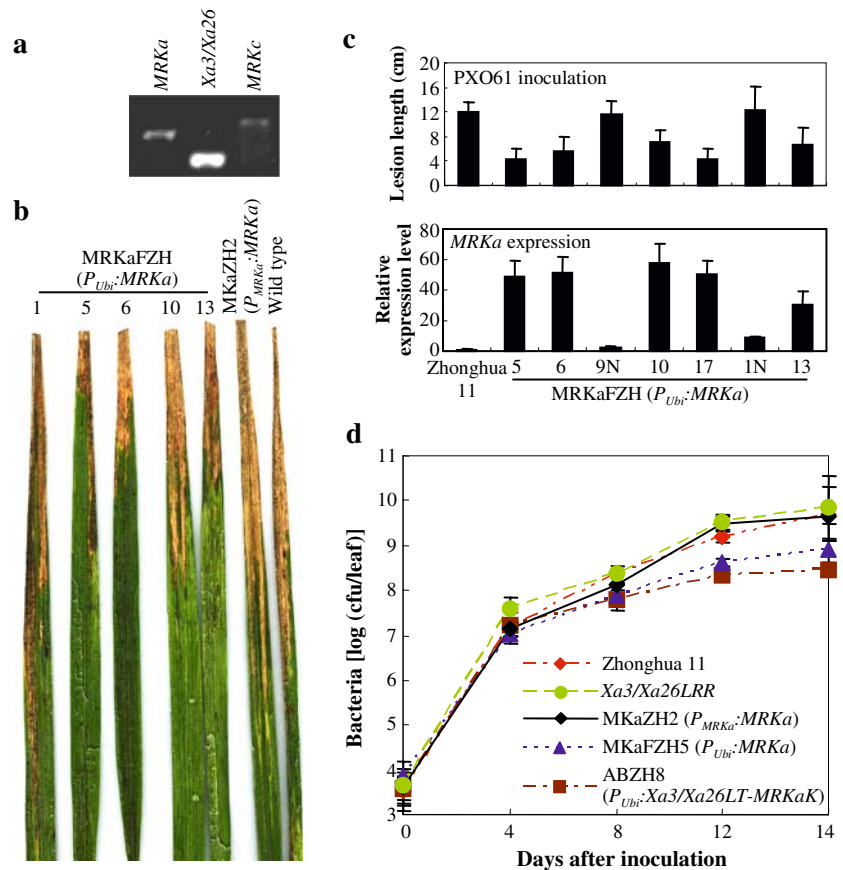
Chimeric but not truncated member of *Xa3/Xa26* family mediates partial resistance to *Xoo*

Rice bacterial blight resistance gene *Xa21* also encodes similar LRR receptor kinase as XA3/XA26 (Song et al. 1995). This type of proteins consists of three conserved structures: an extracellular LRR domain, a transmembrane (TM) region, and a cytoplasmic kinase domain (Song et al. 1995; Sun et al. 2004; Torii 2004). One of the *Xa21* family members, *Xa21D*, encodes a truncated LRR receptor kinase lacking the kinase domain; XA21D confers moderate resistance to *Xoo* as compared with XA21 (Wang et al. 1998). To determine whether truncated XA3/XA26 also confer resistant to *Xoo*, we transferred the truncated *Xa3/Xa26*, *Xa3/Xa26LT* encoding LRR-TM (corresponding to the amino acid position 1 to 795 of XA3/XA26) under the regulation of P_{Ubi} (Fig. 2a) into rice cultivar Zhonghua 11. A total of 16 independent positive transgenic plants named as 26LZH were obtained and inoculated with PXO61. All transgenic plants were susceptible as the wild type (Fig. 2b).

We then introduced a chimeric gene, *Xa3/Xa26LT-MRKaK* that was composed of the DNA fragment encoding the LRR domain and TM region of *Xa3/Xa26* (Xa3/Xa26LT) and the DNA fragment encoding the kinase domain of *MRKa* (MRKaK) and controlled by P_{Ubi} promoter, into Zhonghua 11 (Fig. 2a). Twenty-nine independent positive transformants named ABZH were obtained (Supplemental Table 2). Twenty-three of the 29 T_0 plants carrying *Xa3/Xa26LT-MRKaK* showed significantly enhanced ($P < 0.05$) resistance to *Xoo* strain PXO61 with the lesion length ranged from 3.5 ± 0.4 to 7.4 ± 1.2 cm as compared with 10.2 ± 1.4 cm of the wild-type Zhonghua 11 (Fig. 2b). The enhanced resistance was associated with expression of *Xa3/Xa26LT-MRKaK* in the T_0 plants (Fig. 2c). T_1 families derived from two of the resistant T_0 plants, ABZH8 and ABZH21, were further examined individually. The enhance resistance co-segregated with *Xa3/Xa26LT-MRKaK* in the two T_1 families (Supplemental Table 2). The growth rate of PXO61 on resistant T_1 plants carrying *Xa3/Xa26LT-MRKaK* at the booting stage was 33.2-fold lower than that on wild type at 14 days after inoculation (Fig. 1d). These results suggest that the improved resistance of the transgenic plants was due to the existence of *Xa3/Xa26LT-MRKaK*. The average lesion length of T_1 transgenic plants carrying the chimeric gene was approximately 6.2 cm longer than the plants carrying *Xa3/Xa26* in the same genetic background after PXO61 infection, suggesting that overexpression of *Xa3/Xa26LT-MRKaK* only mediated partial resistance to *Xoo*.
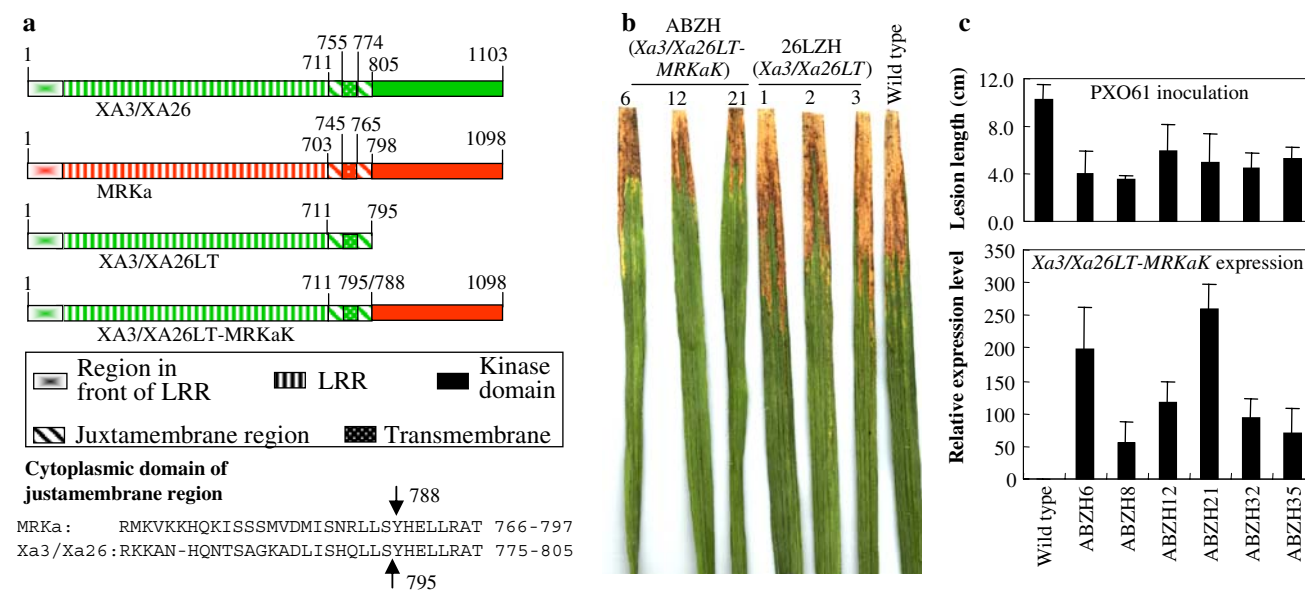
a Predicted structures encoded by *Xa3/Xa26*, *MRKa*, *Xa3/Xa26LT*, and *Xa3/Xa26LT-MRKaK*

Cytoplasmic domain of justamembrane region

MRKa: RMKVKKHQKISSSMVDMISNRLLSYHELLRAT 766-797
Xa3/Xa26:RKKAN-HQNTSAGKADLISHQLLSYHELLRAT 775-805

Fig. 2 Performance of transgenic plants carrying truncated *Xa3/Xa26* gene (*Xa3/Xa26LT*) and chimeric gene (*Xa3/Xa26LT-MRKaK*) of *Xa3/Xa26* and *MRKa*. Zhonghua 11 is wild type. **a** Predicted structures encoded by *Xa3/Xa26*, *MRKa*, *Xa3/Xa26LT*, and *Xa3/Xa26LT-MRKaK* (Sun et al. 2004, 2006). The juxtamembrane region was determined according to He et al. (2000). *LRR* leucine-rich repeat. **b** Leaves from T_0 transgenic plants carrying P_{Ubi}:*Xa3/Xa26LT* or P_{Ubi}:*Xa3/Xa26LT-MRKaK* at 14 days after inoculation with *Xoo* strain PXO61. **c** Enhanced resistance to PXO61 was associated with expression of *Xa3/Xa26LT-MRKaK* in T_0 transgenic plants analyzed by RT-qPCR

Suppressing *Xa3/Xa26* in Minghui 63 results in susceptibility

To further determine that *MRKa* and *MRKc* do not have bacterial resistance function when regulated by their native promoters, we suppressed *Xa3/Xa26* expression by using the RNAi strategy in rice cultivar Minghui 63. Minghui 63 is moderately resistant to *Xoo* strain PXO61 (Yang et al. 2003). Fifteen independent positive transformants named Xa26DS were obtained (Supplemental Table 2). Eleven of the 15 transgenic plants carrying *Xa3/Xa26*-RNAi construct showed significantly increased ($P < 0.05$) susceptibility to PXO61 with the lesion length ranged from 11.6 ± 2.8 to 16.3 ± 3.1 cm as compared with 8.7 ± 2.3 cm of wild-type Minghui 63. The susceptible transgenic plants showed approximately 40–93% reduced *Xa3/Xa26* transcripts compared to the wild type (Fig. 3), which suggested that the susceptibility of the transgenic plants was associated with the suppression of *Xa3/Xa26*.

The sequence of the DNA fragment of *Xa3/Xa26* used for construction of the RNAi construct had the most diversity to the sequences of *MRKa* and *MRKc* compared to the rest sequence of *Xa3/Xa26*. But this fragment still had 73 and 53% sequence identity to *MRKa* and *MRKc*, respectively. Some of the *Xa3/Xa26*-suppressing plants also showed repressed expression of *MRKa* and *MRKc* (Fig. 3). However, the susceptible transgenic plants Xa26DS4 and Xa26DS5 showed no significant suppression ($P > 0.05$) of
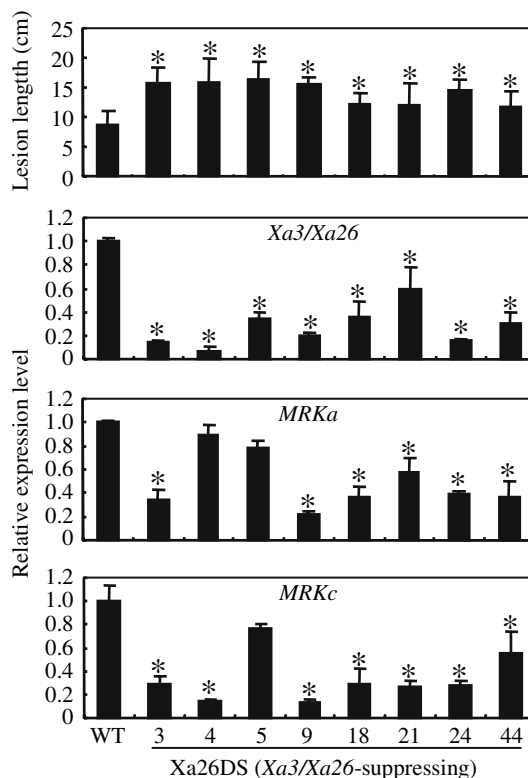

Fig. 3 The susceptibility of the transgenic plants (Xa26DS) was associated with the suppression of *Xa3/Xa26* but not associated with the suppression of *MRKa* or *MRKc* analyzed by RT-qPCR. *Asterisks* indicate a significant difference ($P < 0.05$) between the transgenic plants and wild type Minghui 63 (WT)

MRKa and/or *MRKc*. In addition to that transgenic plants carrying *MRKa* or *MRKc* regulated by its native promoter were susceptible to *Xoo*, our previous fine mapping data have revealed that the *Xoo* resistance of rice cultivar Minghui 63 is controlled by a single gene in the *Xa3/Xa26* locus (Yang et al. 2003). These results suggest that *MRKa* and *MRKc* may not mediate bacterial resistance in *indica* cultivar Minghui 63 when regulated by their native promoters.

MRKa and *MRKc* have the similar expression pattern as *Xa3/Xa26*

The DNA fragment harboring the promoter of *Xa3/Xa26* in Minghui 63, P_{Xa26}, was fused with reporter genes encoding GFP and GUS, respectively. The expression of reporter proteins was only detected in the vascular systems of root, leaf, sheath, stem, and filament of floret (Fig. 4). Tissue structural analysis showed that reporter proteins were preferentially expressed in the parenchyma cells surrounding the vascular vessels in leaf, sheath, stem, and root; the reporter proteins were also detected in the sclerenchyma cells of the vascular bundle sheath in the stem (Fig. 4).

The DNA fragments harboring the promoters of *MRKa* and *MRKc* form Minghui 63 were fused with GFP, respectively. Examination of the plants carrying P_{MRKa}:*GFP* or P_{MRKc}:*GFP* showed that the reporter protein driven by P_{MRKa} or P_{MRKc} had the similar expression pattern as the reporter protein driven by P_{Xa26} in root, leaf, sheath, stem, and floret; the reporter proteins were only detected in the vascular systems of these tissues (Fig. 4). However, reporter protein driven by P_{MRKc} was also detected in the vascular systems of lemma and palea of floret in addition to the filament as compared to that driven by P_{MRKa} and P_{Xa26} (Fig. 4). These results indicate that *Xa3/Xa26*, *MRKa* and *MRKc* are specially expressed in the cells surrounding the vascular vessels.

Discussion

The present results suggest that among the three intact genes of *Xa3/Xa26* family in rice cultivar Minghui 63, *Xa3/Xa26*, *MRKa* and *MRKc*, only *Xa3/Xa26* mediate disease resistance to *Xoo* when regulated by their native promoters. However, *MRKa* but not *MRKc* can mediate partial resistance to *Xoo* when regulated by a constitutive strong promoter. These results suggest that *MRKa* has dosage effect in bacterial resistance. It is generally accepted that the LRR domain of some type of R proteins is the major contributor of pathogen recognition specificity (Dangl and Jones 2001). Evolutionary analysis shows that most of the positive selected sites of *Xa3/Xa26* family locate in the LRR domain, indicating the importance of LRR domain in the
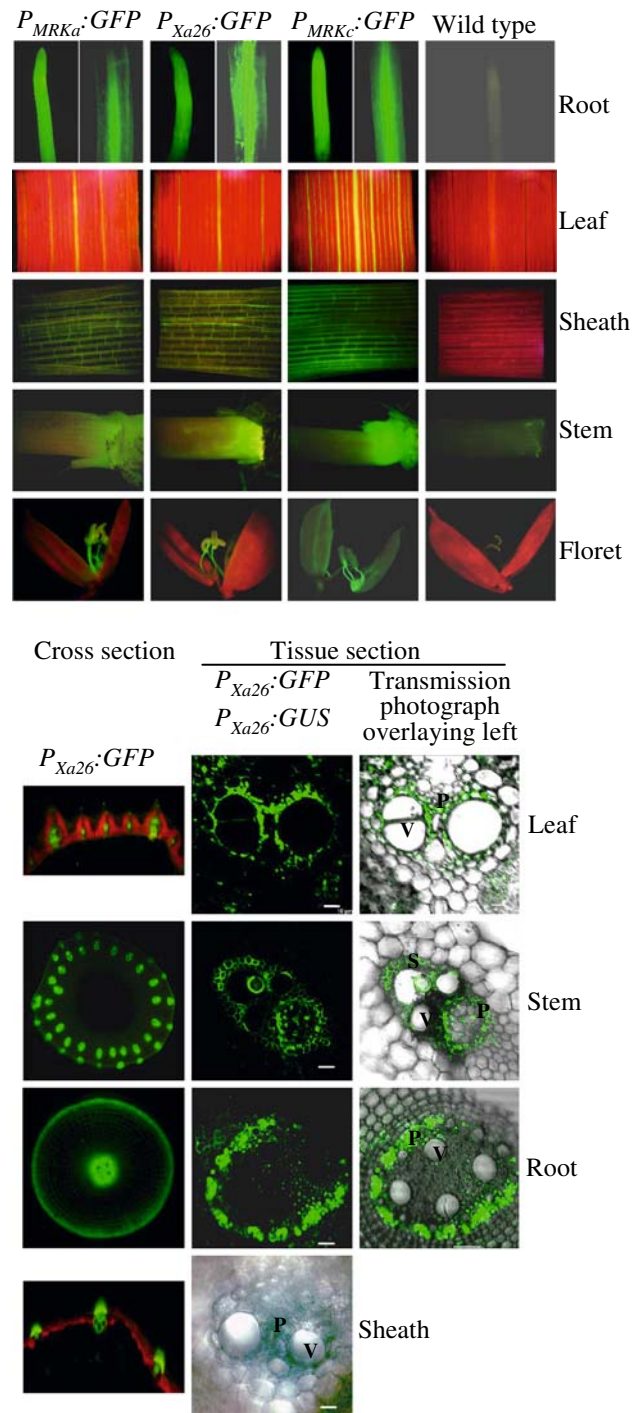

Fig. 4 Expression patterns of P_{Xa26}:*GFP/GUS*, P_{MRKa}:*GFP* and P_{MRKc}:*GFP* in different tissues. *P* parenchyma cells; *S* sclerenchyma cells; *V* vascular element; bar, 10 μm

function of this family (Sun et al. 2006). The LRR domain of MRKa share 69% amino acid sequence identity and 82% sequence similarity with XA3/XA26. The sequence diversity may result in that MRKa cannot recognize bacterial effector or guardees, the pathogenicity targets of the host, when expressed at low level. However, large amounts of

MRKa proteins may facilitate no perfect interaction between host and pathogen, which resulted in enhanced resistance to *Xoo* in the *MRKa*-overexpressing plants. *MRKa* could not confer full resistance to *Xoo*, indicating that overexpression of *MRKa* caused only a partial, but not complete, loss of recognition specificity to *Xoo*. This hypothesis can be also supported by the performance of *MRKc* in rice resistance. The LRR domain of MRKc share 54% amino acid sequence identity and 71% sequence similarity with XA3/XA26. Overexpression of *MRKc* cannot enhance rice resistance to *Xoo*.

Several *R* genes encoding proteins consisted of an extracellular LRR domain and a transmembrane region have been identified from tomato and sugar beet (Jones et al. 1994; Dixon et al. 1996; Cai et al. 1997; Thomas et al. 1997; Dixon et al. 1998). Rice *Xa21* family member, *Xa21D* encoding a truncated LRR receptor kinase lacking the kinase domain, can mediate partial resistance to *Xoo* (Wang et al. 1998). However, the present results indicate that kinase domain is required for the function of *Xa3/Xa26* in bacterial blight resistance. Rice plants carrying truncated *Xa3/Xa26* that lacks the DNA fragment encoding the kinase domain are susceptible to *Xoo* as the wild type. The kinase domain of MRKa can partially restore the function of the truncated XA3/XA26; plants carrying *Xa3/Xa26LT-MRKaK* regulated by maize ubiquitin gene promoter are partially resistance to *Xoo* as compared to *Xa3/Xa26*-mediated resistance. The kinase domain of MRKa share 85% amino acid sequence identity and 91% sequence similarity with XA3/XA26. Most of the amino acid divergence between the two sequences occurs in the conserved motifs of the kinase domains (Fig. 5). Protein kinases transfer signals by phosphorylation of downstream proteins in cellular transduction pathways. The present results suggest that the kinase domain of *Xa3/Xa26* family also influence its function in disease resistance. The kinase domain of XA3/XA26 appears to better facilitates signal transduction than that of MRKa in bacterial resistance. This result is consistent with

previous report that mutation of the kinase domain of rice R protein XA21 impairs XA21-mediated resistance (Andaya and Ronald 2003). Thus, the non-specific interaction between the LRR domain of MRKa and bacterial elicitor or host guarded and the impaired defense signal transduction initiated by the kinase domain of MRKa may explain why *MRKa*-overexpressing plants can only mediate partial resistance to *Xoo*.

Systematic study of the expression patterns of the *R* gene family members is rarely reported. Rice blast resistance gene *Pib*, encoding the NBS-LRR type of protein, belongs to a multigene family. *Pib* was induced by multiple environment signals such as dark, light and pathogen (Wang et al. 2001), and its family members also showed similar expression patterns after treatment under different environment conditions (Wang et al. 2001). The present results show that *MRKa* and *MRKc* have similar expression pattern as *Xa3/Xa26*, which expresses only in the vascular systems of different tissues. The expressional characteristic of *MRKa* and *MRKc* fits the function for the genes conferring resistance to *Xoo* perfectly. *Xoo* is a vascular pathogen, which invades rice plants through hydathodes or wounds, spread throughout the plant via the vascular system, and lives in the vascular vessels. The preferential expression of *R* gene in the vascular tissues provides rapid pathogen recognition. However, *MRKa* and *MRKc* cannot mediate resistance to *Xoo* when they are regulated by their native promoters, suggesting that they are not *R* genes for bacterial blight resistance.

Plant LRR receptor kinases belong to a superfamily. Over 350 members have been identified in rice (Shiu et al. 2004). Most of the characterized plant LRR receptor kinases are involved in regulation of a wide cultivar of development (Torii 2004). Rice plants overexpressing *MRKa*, *MRKc* or the chimeric gene *Xa3/Xa26LT-MRKaK* did not show obvious morphological and developmental modification, implicating that the two genes may be also not involved in developmental regulation.

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MRKa      DNFSYDNMLGAGSFGKVYKGQLSSGLVVAIKVIHQHLEHAMRSFDTECHVLRMARHRNLI  857
XA3/XA26  DDFSDDSMLGFGSFGKVFRGRLSNGMVVAIKVIHQHLEHAMRSFDTECRVLRMARHRNLI  867
                I                    II              III
MRKa      KILNTCSNLDFRALVLEYMPNGSLEALLHSEGRMQLGFLERVDIMLDVSMAMEYLHHEHH  917
XA3/XA26  KILNTCSNLDFRALVLQYMPKGSLEALLHSEQGKQLGFLERLDIMLDVSMAMEYLHHEHY  927
                IV              V                              VI
MRKa      EVVLHCDLKPSNVLLDDDMTAHVSDFGIARLLLGDDSSMISASMPGTVGYMAPEYGALGK  977
XA3/XA26  EVVLHCDLKPSNVLFDDDMTAHVADFGIARLLLGDDNSMISASMPGTVGYMAPEYGTLGK  987
                             VII                    VIII
MRKa      ASRKSDVFSYGIMLLEVFTGKRPTDAMFVGELNIRQWVYQAFPVELVHVLDTRLLQD--C  1035
XA3/XA26  ASRKSDVFSYGIMLLEVFTAKRPTDAMFVGELNIRQWVQQAFPAELVHVVDCQLLQDGSS  1047
                      IX
MRKa      SSPSSLHGFLVPVFELGLLCSADSPEQRMVMSDVVVTLKKIRKDYVKSISTTGSVALPAY  1095
XA3/XA26  SSSSNMHDFLVPVFELGLLCSADSPEQRMAMSDVVLTLNKFRKDYVKMATTVSVV-----  1101
                X                                        XI
MRKa      TKE  1098
XA3/XA26  -QQ  1103
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Fig. 5 Alignment of the kinase domains of XA3/XA26 and MRKa. The conserved subdomains are numbered and underlined according to Hanks et al. (1988). The *solid black shade* indicates different amino acid residues and the *grey shade* indicates residues with similarity. The numbers on the right indicate amino acid positions in XA3/XA26 and MRKa, respectively

Our previous study suggests that *Xa3/Xa26* family may have been subject to a rapid evolution; point mutations with positive selection are a major force during the evolution of this family (Sun et al. 2006). Thus *MRKa* and *MRKc* may be once effective genes for *Xoo* resistance. However, their expressional characteristic and sequence similarity to *Xa3/Xa26* will provide templates for generating novel recognition specificity to face the evolution of *Xoo*. In addition, it is needed to examine whether *MRKa* and *MRKc* are involved in the resistance to other types of rice pathogens.

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