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The Expression Pattern of a Rice Disease Resistance Gene *Xa3/Xa26* Is Differentially Regulated by the Genetic Backgrounds and Developmental Stages That Influence Its Function

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ABSTRACT

Genetic background and developmental stage influence the function of some disease resistance (*R*) genes. The molecular mechanisms of these modifications remain elusive. Our results show that the two factors are associated with the expression of the *R* gene in rice *Xa3* (also known as *Xa26*)–mediated resistance to *Xanthomonas oryza*e pv. *oryzae* (*Xoo*), which in turn influences the expression of defense-responsive genes. The background of *japonica* rice, one of the two major subspecies of Asian cultivated rice, facilitates the function of *Xa3* more than the background of *indica* rice, another rice subspecies. *Xa3* expression gradually increases from early seedling stage to adult stage. *Japonica* plants carrying *Xa3* regulated by the native promoter showed an enlarged resistance spectrum (i.e. resistance to more *Xoo* races), increased resistance level (i.e. further reduced lesion length), and whole-growth-stage resistance compared to the *indica* rice; this enhanced resistance was associated with an increased expression of *Xa3* throughout the growth stages in the *japonica* plants, which resulted in enhanced expression of defense-responsive genes. Overexpressing *Xa3* with a constitutive strong promoter further enhanced rice resistance due to further increased *Xa3* transcripts in both *indica* and *japonica* backgrounds, whereas regulating *Xa3* with a pathogen-induced weak promoter impaired rice resistance.

Plant disease resistance (*R*) genes are an important source of plant immunity. The encoding products of *R* genes recognize or guard against specific pathogen effectors and trigger signal transduction cascades that lead to rapid disease resistance in the host plants (Dangl and Jones 2001; Belkhadir *et al.* 2004). Since each R protein can only directly or indirectly recognize limited types of pathogen effectors, *R* genes are characterized to mediate race-specific resistance. The same plant species carrying different *R* genes frequently has a different resistance spectrum to different pathogen species or the same pathogen species but different races. A large number of R proteins have been identified as recognizing different pathogens, including bacteria, fungi, viruses, oomycetes, and nematodes, from diverse plant species; most of the characterized proteins contain a leucine-rich repeat (LRR) domain (Martin *et al.* 2003). It is generally accepted that the LRR domain of the LRR-containing R proteins is the major contributor of pathogen recognition specificity (Dangl and Jones 2001). A few studies have revealed that non-LRR regions, such as the Toll/interleukin-1 receptor homology region and the region between signal peptide and LRR domain of some R proteins, are also involved in pathogen resistance specificity (Ellis *et al.* 1999; Luck *et al.* 2000; Van der Hoorn *et al.* 2001).

Although the amino acid sequence of R proteins is an important determinant of pathogen resistance specificity, limited information has shown that other host factors are also required for pathogen recognition in some *R* gene–mediated disease resistances. Host genetic background is a factor that influences the function of *R* genes. The rice *Xa26* gene conferring resistance to *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), which causes bacterial blight, the most devastating plant bacterial disease worldwide, is one example. Asian-cultivated rice (*Oryza sativa* L.) consists of two major groups, which are known

by the subspecies names *indica* and *japonica*. Transgenic plants carrying *Xa26* in the background of *japonica* variety Mudanjiang 8 showed increased resistance to five *Xoo* strains and enhanced resistance to another three *Xoo* strains as compared to the gene donor of *indica* variety Minghui 63 (Sun *et al.* 2004). In addition, different *indica* backgrounds also influence the function of *R* genes. Minghui 63 carries another bacterial blight resistance gene, *Xa25(t)*, in addition to *Xa26* (Chen *et al.* 2002). Another *indica* rice line IRBB3 is well known to carry only one *R* gene, *Xa3*, for *Xoo* resistance (Ogawa *et al.* 1991). Our studies have demonstrated that *Xa3* and *Xa26* are the same gene, with identical sequences in the coding region and only one nucleotide substitution occurring at 475-bp upstream of the translation initiation site (Sun *et al.* 2004; Xiang *et al.* 2006). Thus this gene is named *Xa3*. However, IRBB3 showed better resistance to different *Xoo* strains than Minghui 63 (Sun *et al.* 2004), although this difference may be partly caused by different quantitative trait loci for disease resistance in the two genetic backgrounds. Furthermore, the function of an allele of *Arabidopsis R* gene *RPS2* is influenced by genetic background, and the LRR domain determines the effectiveness of the interaction between RPS2 and other host factors in *RPS2*-mediated resistance (Banerjee *et al.* 2001).

The developmental stage of the host is another factor that influences the function of *R* genes. The activity of rice bacterial blight resistance gene *Xa21* is developmentally controlled. *Xa21*-mediated resistance increases progressively from the susceptible juvenile stage to full resistance at the later adult stage (Century *et al.* 1999). Several other rice *R* genes conferring resistance to *Xoo* also mediate full disease resistance only in the adult stage (Zhang and Mew 1985; Mew 1987; Goel and Gupta 1990; Ogawa 1993). Developmentally controlled disease resistance has also been observed in other

plant-pathogen systems. The *Cf-9B* is a family member of the tomato *Cf-9* gene, conferring resistance to *Cladosporium fulvum*; *Cf-9B* mediates weaker resistance than *Cf-9* and protects only mature plants from infection (Panter *et al*. 2002).

Although different host factors can modify the function of *R* genes, the molecular mechanisms of these modifications remain elusive. Here we report that the expression pattern of rice *Xa3*, encoding LRR receptor kinase type of protein, is associated with its variant resistant activity in different genetic backgrounds and different developmental stages. A higher expression level of *Xa3* results in a wider resistance spectrum, strong resistance level, and whole-growth-stage resistance. The explanation of the dosage-dependent resistance conferred by *Xa3* is discussed. *Xa3* may be used as a tool to unravel the molecular mechanisms of R protein function in genetic background–dependent and developmental stage–dependent disease resistance. In addition, *Xa3*-overexpressing plants showed no remarkable morphologic and developmental difference from wild type, implicating the gene's value in breeding programs.

MATERIALS AND METHODS

Plant transformation: The overexpression construct carrying P_{Ubi}*:Xa3* was made by inserting the genomic fragment of *Xa3* coding region amplified using primers MRKbR and MRKbF (Supplemental Table 1) into vector pU1301 (Qiu et al. 2007) (Supplemental Figure 1A). The construct carrying P_{WRKY13}*:Xa3* was made by inserting *Xa3* coding region amplified using primers MRKbR and MRKbF into vector pI1381 (Supplemental Figure 1B). The pI1381 was modified by insertion of a 728-bp rice *WRKY13* gene promoter locating at –691 to +37 of *WRKY13* (Qiu et al. 2007) into the

multiple cloning sites of vector pCAMBIA1381. The construct (MKb) carrying P_{Xa3}*:Xa3* was the same used previously (Supplemental Figure 1C, Sun *et al.* 2004). *Agrobacterium*-mediated transformation was performed according to the protocol of Lin and Zhang (2005).

Pathogen inoculation: To evaluate bacterial blight disease, plants were inoculated with *Xoo* strains at the seedling or booting stage, as described previously (Sun *et al.* 2004). Z173 is a Chinese strain. PXO61, PXO86, PXO79, PXO71, PXO99, PXO145, and PXO280 are strains representing Philippine races 1, 2, 3, 4, 6, 7, and 8, respectively. T7174, T7147, and T7133 are Japanese strains. Disease was scored by measuring the percent lesion area (lesion length/leaf length) at 2–3 weeks after inoculation. Mock-inoculated (control) plants were treated under the same conditions, except that pathogen suspension was replaced with water.

Reverse transcription (RT)–quantitative PCR analysis (qPCR): RT-PCR was conducted as described by Wen et al. (2003). Quantitative PCR was performed using primers RealF and Real2R for the plants in the Zhonghua 11 background and using primers RKb3F and RealR for the plants in the backgrounds of Mudanjiang 8, 02428, Minghui 63, or IRBB3 (Supplemental Table 1) and 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

***Japonica* background facilitates the function of *Xa3*:** Our previous study has shown that *Xa3* (also known as *Xa26*) mediates race-specific resistance to *Xoo*; transgenic rice plants carrying *Xa3* in the genetic background of *japonica* rice variety Mudanjiang 8 showed enhanced resistance as compared with a *Xa3* donor, *indica* rice variety Minghui 63 (Sun *et al.* 2004). To explore whether this is a general phenomenon, we transferred the *Xa3* gene with its native promoter (P_{Xa3}) from Minghui 63 to two other susceptible *japonica* rice varieties, Zhonghua 11 and 02428. A total of 12 independent transformants (MKbZH) in Zhonghua 11 background were generated. Eight of the nine positive transgenic plants were highly resistant to *Xoo* strain PXO61 at adult (booting) stage, with lesion areas ranging from 0.4 ± 0.2% to 3.3 ± 2.0%, as compared to 39.0 ± 11.9% and 25.6 ± 4.5% measured for the controls of susceptible Zhonghua 11 and moderately resistant Minghui 63, respectively (Figure 1A, Supplemental Table 2). The bacterial growth analysis demonstrated that the growth rate of PXO61 on resistant transgenic plants at the booting stage was 87-fold lower than that on wild type (Figure 1B). T_1 families derived from three of the resistant T_0 plants carrying one copy of *Xa3* 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 resistance cosegregated with *Xa3* in all three T_1 families (Supplemental Table 2), indicating that the improved resistance was due to the existence of *Xa3*. Resistant T_1 plants from the MKbZH2 family were further examined for their resistance spectrums. Transgenic plants showed significantly enhanced ($P < 0.01$) resistance to five (PXO61, PXO86, PXO79, PXO71, and PXO145) of the seven

strains representing different *Xoo* races compared with wild type and Minghui 63 at booting stage (Table 1). The transgenic plants were also more resistant than transgenic line Rb17-2 carrying one copy of P_{Xa3}*:Xa3* in the background of *japonica* Mudanjiang 8 (Table 1).

Two independent positive transformants (MKb024) in the background of *japonica* variety 02428 were obtained. The T_0 transgenic plants were highly resistant to *Xoo* strain PXO61, with lesion areas of 3.1 ± 0.3% and 3.2 ± 0.2%, as compared to 47.5 ± 9.4% and 23.9 ± 2.2% measured for the controls of susceptible 02428 and moderately resistant Minghui 63 at booting stage, respectively (Figure 1A, Supplemental Table 2). The resistance of the T_1 family cosegregated with *Xa3* (Supplemental Table 2), indicating that the improved resistance was due to *Xa3*. These results suggest that genetic background has a large influence on the function of *Xa3* and that a *japonica* background facilitates *Xa3* function more than an *indica* background.

Host background–enhanced resistance is associated with increased expression of *Xa3*: To determine whether genetic background influenced the expression of *Xa3*, we quantified its transcripts in different rice lines by RT-qPCR. In addition to Minghui 63, the *indica* line IRBB3 also carries the *Xa3* gene (Sun *et al.* 2004; Xiang *et al.* 2006). Transgenic lines Rb1, Rb49, and Rb17-2, carrying one copy of *Xa3* driven by a native promoter in the genetic background of *japonica* Mudanjiang 8, showed enhanced resistance compared with the native rice lines carrying *Xa3* (Sun *et al.* 2004). Compared with Minghui 63 and IRBB3, transgenic lines carrying one copy of *Xa3* driven by a native promoter in the genetic backgrounds of Zhonghua 11 and Mudanjiang 8 showed 3- to 242-fold more *Xa3* transcripts (Figure 2A). Transgenic plants carrying P_{Xa3}*:Xa3* in the 02428 background had 44-fold more *Xa3* transcripts. The data also showed that the

expression level of *Xa3* was remarkably higher in the Zhonghua 11 background than in Mudanjiang 8 and 02428 backgrounds (Figure 2A). These results suggest that increased *Xa3* transcripts may be associated with the enhanced resistance in the transgenic plants with a *japonica* background.

To evaluate the above hypothesis, we transferred *Xa3* driven by a strong constitutive promoter, maize ubiquitin gene promoter (P_{Ubi}), into Zhonghua 11, Mudanjiang 8, and Minghui 63. Eight of the 10 positive T_0 plants, all 10 positive T_0 plants, and 11 of the 12 positive T_0 plants transformed with P_{Ubi}*:Xa3* in backgrounds of Zhonghua 11, Mudanjiang 8, and Minghui 63, respectively, were highly resistant to PXO61 at booting stage (Figure 1A, Supplemental Table 2). The lesion area of these highly resistant plants ranged from 0.2 ± 0.1% to 1.5 ± 0.5% in the Zhonghua 11 background as compared to 39.0 ± 11.9% and 25.6 ± 4.5% measured for the susceptible wild type and moderately resistant Minghui 63 controls, respectively; and from 2.0 ± 0.4% to 8.4 ± 0.6% in Minghui 63 background as compared to 53.2 ± 2.8 measured for the moderately resistant wild type. Similar results were also obtained for the transgenic plants carrying P_{Ubi}*:Xa3* in Mudanjiang 8 background (Supplemental Table 2). T_1 families derived from two to three of the resistant T_0 plants from each genetic background were further examined. It was shown that the resistance cosegregated with *Xa3* in all the T_1 families examined (Supplemental Table 2), indicating that the enhanced resistance was due to the transgene *Xa3*.

Resistant T_1 plants MKbFZH2 carrying P_{Ubi}*:Xa3* in Zhonghua 11 background were further examined for their resistance spectrums. Transgenic plants showed significantly enhanced ($P < 0.01$) resistance to all seven *Xoo* strains compared with wild type (Table 1). The MKbFZH2 plants also appeared to be more resistant to *Xoo* strains

PXO61, PXO79, PXO71, PXO99, PXO145, and Z173 than transgenic plants MKbZH2 carrying P_{Xa3}*:Xa3*, as determined by a comparison of lesion areas at booting stage (Table 1). A bacterial growth analysis also indicated that plants carrying P_{Ubi}*:Xa3* were more resistant to *Xoo* infection than plants carrying P_{Xa3}*:Xa3* in Zhonghua 11 background; the bacterial growth rate of PXO61 on MKbFZH plants was 1.5-fold lower than that on MKbZH plants at 14 days after inoculation (Figure 1B). Similar results were also obtained in transgenic plants in Mudanjiang 8 background. Resistant T_1 plants MKbFMDJ4, MKbFMDJ5, and MKbFMDJ7 carrying P_{Ubi}*:Xa3* appeared to be more resistant to strains PXO61, PXO79, PXO71, PXO145, PXO280, T7174, T7147, T7133, and Z173 than transgenic line Rb17-2 carrying P_{Xa3}*:Xa3*, as determined by a comparison of lesion areas at booting stage (Table 2). The bacterial growth rate of PXO61 on MKbFMDJ plants was also 1.5-fold lower than that on Rb17-2 plants at 14 days after inoculation (Figure 1B). Overexpression of *Xa3* enhanced rice resistance not only in plants in *japonica* background but also in those in *indica* background. Transgenic plants MKbFMH2, MKbFMH3, MKbFMH4, MKbFMH6, and MKbFMH7 carrying P_{Ubi}*:Xa3* in Minghui 63 background showed significantly enhanced ($P < 0.01$) resistance to six (PXO61, PXO79, PXO71, PXO145, T7133, and Z173) of the seven *Xoo* strains as compared to the donor of *Xa3*, Minghui 63, at booting stage (Table 2).

We also transferred *Xa3* driven by a weak and pathogen-induced promoter, rice *OsWRKY13* gene promoter (P_{WRKY13}) (Qiu *et al.* 2007), into Zhonghua 11 and Mudanjiang 8. All 16 and 4 positive T_0 plants in Zhonghua 11 and Mudanjiang 8 backgrounds, respectively, carrying P_{WRKY13}*:Xa3* showed enhanced resistance as compared to wild type; this enhanced resistance cosegregated with *Xa3* in T_1 families (Figure 1A, Supplemental Table 2). However, *Xa3*-mediated resistance was significantly

impaired ($P < 0.01$) in plants carrying P_{WRKY13}:*Xa3* as compared to plants carrying P_{Xa3}:*Xa3* in the same genetic background. The average lesion area of transgenic plants carrying P_{WRKY13}:*Xa3* was approximately 3- to 11-fold larger than that of the transgenic plants carrying P_{Xa3}:*Xa3* in the same genetic background on infection (Table 3). The bacterial growth rate on plants carrying P_{WRKY13}:*Xa3* was 7.9- and 29.5-fold higher than that on plants carrying P_{Xa3}:*Xa3* in Zhonghua 11 and Mudanjiang 8 backgrounds, respectively (Figure 1B).

The *Xa3* expression level driven by P_{Ubi} was, on average, 2-, 11-, and 63-fold higher than that driven by the native promoter in the backgrounds of Zhonghua 11, Mudanjiang 8, and Minghui 63, respectively. The *Xa3* expression level driven by P_{WRKY13} was only, on average, 2% and 18% of that driven by the native promoter in the backgrounds of Zhonghua 11 and Mudanjiang 8, respectively (Figure 2A). The negative correlation between lesion area and *Xa3* expression level in the plants shown in Figure 2A was -0.523, significant at $\alpha = 0.05$ ($n = 17$). The variable resistance ability of plants carrying *Xa3* driven by different promoters and different expression levels of *Xa3* suggest that the function of *Xa3* is associated with its expression level: The higher its expression, the more resistant the plant.

Developmentally controlled *Xa3* activity is associated with its expression level: Minghui 63 and IRBB3 were susceptible to *Xoo* strains PXO61 and PXO71 at seedling stage (Table 4). However, Minghui 63 became moderately resistant or moderately susceptible to PXO61, although still susceptible to PXO71, and IRBB3 became resistant to PXO61 and PXO71 at adult (booting) stage (Tables 1 and 2, Sun *et al.* 2004). Plants carrying P_{Xa3}:*Xa3* in the background of *japonica* variety Mudanjiang 8 were highly resistant to these *Xoo* strains at seedling stage (Table 4, Sun et al. 2004). Transgenic

plants carrying P_{Xa3}:*Xa3* in Zhonghua 11 and 02428 backgrounds were also highly resistant to *Xoo* strains at seedling (four-leaf) stage as compared to Minghui 63 and IRBB3 (Table 4). The growth rates of PXO61 on resistant transgenic plants carrying P_{Xa3}:*Xa3* in Zhonghua 11, Mudanjiang 8, and 02428 backgrounds were 28-, 16-, and 13-fold lower than those on Minghui 63 at 12 days after bacterial infection at the four-leaf stage, respectively (Supplemental Figure 2, Figure 1C). The bacterial growth rates on transgenic plants carrying P_{Ubi}:*Xa3* in Zhonghua 11, Mudanjiang 8, and Minghui 63 backgrounds were 25-, 44-, and 101- to 207-fold lower than the growth rate on Minghui 63 at the four-leaf stage, respectively.

To examine whether *Xa3* is expressed differentially at different developmental stages, Minghui 63 and transgenic line Rb49 carrying P_{Xa3}:*Xa3* in the background of *japonica* variety Mudanjiang 8 were grown with staggered planting so that RNA samples were obtained from plants at the two-leaf, four-leaf, maximum-tillering, booting, and grain-filling stages at the same time from different varieties. RT-qPCR analysis showed that *Xa3* expression level was very low at the two-leaf stage, gradually increased with development, and reached the highest level at the maximum-tillering or booting stage in both Minghui 63 and Rb49 (Figure 2B). However, *Xa3* transcripts in Rb49 were approximately 21-, 11-, 12-, 17-, and 5-fold higher than those in Minghui 63 from the two-leaf to the grain-filling stages, respectively. The association between increasing *Xa3*-mediated resistance and *Xa3* expression level accompanying development suggests that the developmentally controlled disease resistance in the *indica* background plants is *Xa3*-dosage-dependent.

Increasing *Xa3* expression results in enhanced expression of defense-responsive genes: The expression of rice *OsWRKY13*, encoding a transcription factor, was rapidly

induced in incompatible (resistant) host-pathogen interaction and lightly induced in compatible (susceptible) host-pathogen interaction; overexpression of *OsWRKY13* enhanced rice resistance to *Xoo* (Wen *et al.* 2003; Qiu *et al.* 2007). *NH1* is the rice orthologue of *Arabidopsis NPR1*; this gene was rapidly induced in incompatible host-pathogen interaction as compared to compatible interaction and overexpression of *NH1* enhanced rice resistance to *Xoo* (Chern *et al.* 2005; Yuan *et al.* 2007). These results suggest that *OsWRKY13* and *NH1* are involved in *R* gene-mediated resistance against *Xoo*. To determine the role of *OsWRKY13* and *NH1* in genetic background-influenced and *Xa3*-mediated resistance, we analyzed their expression in rice lines with different expression levels of *Xa3*. RT-qPCR analysis showed that plants with more *Xa3* transcripts (Figure 2A) induced the expression of *OsWRKY13* and *NH1* more rapidly and/or effectively upon bacterial infection (Figure 3). *Japonica* transgenic lines Rb49 and MKbZH1 carrying one copy of P_{Xa3}*:Xa3* had 5.6- and 15-fold more *OsWRKY13* transcripts and 2.1- and 1.6-fold more *NH1* transcripts than *indica* line Minghui 63 carrying *Xa3* as compared with the maximum transcript level within 1 d of post-infection. The same two transgenic lines had 1.2- and 3.1-fold more *OsWRKY13* transcripts and 3.9- and 2.9-fold more *NH1* transcripts than *indica* line IRBB3 carrying *Xa3*. Transgenic line MKbFZH2 carrying strong expression construct P_{Ubi}*:Xa3* had 2-fold more *OsWRKY13* transcripts than MKbZH1 in the same genetic background, although the maximum transcript level of *NH1* in MKbFZH2 was slightly lower than that in MKbZH1. However, the maximum transcript levels of *OsWRKY13* and *NH1* in transgenic line 12IMKbZH2 carrying weak expression construct P_{WRKY13}*:Xa3* were only 59% and 36%, respectively, those in MKbZH1. In consistence with the expression pattern reported previously (Qiu *et al.* 2007; Yuan *et al.* 2007), both resistant and

susceptible responses in the same genetic background induced *OsWRKY13* and *NH1*, but the former reaction resulted more *OsWRKY13* and *NH1* transcripts than the latter as compared with the maximum transcript level within 1 d of post-infection (Figure 3).

Pathogen infection differentially influences *Xa3* expression in plants with different genetic backgrounds: *Xa3* expression was suppressed (approximately 2.5- to 3-fold) at 4 h postinoculation and then induced (approximately 2- to 4-fold) as compared to noninfected plants in *indica* rice lines Minghui 63 and IRBB3 (Figure 4). This suppression was not observed in transgenic lines carrying P_{Xa3}*:Xa3* in *japonica* backgrounds. In contrast, pathogen infection induced (approximately 5.5- to 7-fold) *Xa3* expression in transgenic lines Rb49 and Rb17-2 in the genetic background of *japonica* variety Mudanjiang 8 (Figure 4). The expression level of *Xa3* in transgenic lines MKbZH1 and MKbZH2 in the background of *japonica* Zhonghua 11 showed no remarkable differences before and after pathogen infection. Pathogen infection showed approximately 10-fold induction of *Xa3* in the transgenic plants 12IMKbZH2 carrying one copy of P_{WRKY13}*:Xa3* with a Zhonghua 11 background at 12 h after infection, although the induced transcript level was still approximately 1.5-fold lower than that in noninoculated MKbZH2 plants carrying P_{Xa3}*:Xa3* (Figure 4). These results suggest that genetic background also influences *Xa3* expression in response to bacterial invasion.

DISCUSSION

The present results confirm our previous finding that a *japonica* background facilitates the function of *Xa3* more than an *indica* background (Sun *et al.* 2004). These results are also consistent with the identification and application of *Xa3* in rice production. This gene was first identified in *japonica* variety Wase Aikoku 3 (Ezuka *et*

al. 1975) and is an important resistance gene in *japonica* cultivar breeding in China (Xu *et al.* 2004), one of the largest rice-growing countries in the world.

The resistance spectrum conferred by an *R* gene is related to pathogen recognition specificity. Although the amino acid sequence, especially the LRR sequence, of LRR-containing R proteins is the major determinant of pathogen recognition (Dangl and Jones 2001), our results indicate that the expression level of an *R* gene can also influence the resistance spectrum conferred by this gene. Increasing *Xa3* expression can enlarge the resistance spectrum mediated by *Xa3*. Studies of other genes also support that some *R* gene–mediated resistance has a dosage effect. Overexpression of tomato *R* gene *Pto*, encoding a serine/threonine protein kinase, activates defense responses and confers broad resistance (Tang *et al.* 1999). Overexpression of tomato *Prf* and *Arabidopsis RPS2*, encoding nucleotide-binding site (NBS)-LRR type proteins, leads to constitutive activation of the defense response and broad-spectrum resistance, respectively (Oldroyd and Staskawicz 1998; Tao *et al.* 2000). Overexpression of tomato LRR membrane protein type *R* gene in *Nicotiana benthamiana* also induces a constitutive defense response (Wulff *et al.* 2004). However, not all *R* genes can mediate an enlarged resistance spectrum by overexpression. Overexpression of *Arabidopsis SSI4* gene, encoding Toll interleukin 1 receptor–NBS–LRR protein, failed to enhance disease resistance, while its mutant allele, *ssi4*, encoding a protein with a single amino acid substitution in the NBS domain, showed enhanced resistance to bacterial and oomycete pathogens (Shirano et al. 2002).

The putative mechanisms that enhanced resistance associated with increased expression of *Xa3* include the following. First, increasing XA3 proteins may facilitate their interaction with different pathogen effectors or guardees, the pathogenicity targets

of the host (Dangl and Jones 2001). The interaction specificity between an R protein and pathogen effector or the host guardee should determine the pathogen recognition efficiency of a host. High specific host-pathogen interaction may require only small amounts of R proteins, which may explain why *R* genes usually show a low level of expression (De Ilarduya and Kaloshian 2001; Shen *et al.* 2002; Paal *et al.* 2004; Schornack *et al.* 2004; Sun *et al.* 2004; Huang *et al.* 2005). Otherwise, large amounts of R proteins are needed for nonperfect interaction. It has been reported that overexpression of a pathogen effector avrBs3 causes a loss of recognition specificity of tomato R protein Bs4 (Schornack *et al.* 2004). The enlarged resistance spectrum mediated by *Xa3* in the *japonica* background and in overexpression status may be due to the loss of perception specificity of the XA3 protein to some bacterial effectors. However, plants carrying P_{Ubi}*:Xa3* could not confer full resistance to *Xoo* strains PXO99 and Z173, indicating that overexpression of *Xa3* caused only a partial, but not complete, loss of recognition specificity among different *Xoo* races. Second, increasing XA3 proteins may facilitate more rapid or effective initiation of defense signaling transduction during host-pathogen interaction, which resulted in reduced lesion area and bacterial growth rate. Both *OsWRKY13* and *NH1* are involved in *R* gene-mediated *Xoo* resistance and they are dosage-dependent in bacterial resistance; OsWRKY13 and NH1 are transcript regulators that directly or indirectly control the expression of a subset of defense-responsive genes (Wen *et al.* 2003; Chern *et al.* 2005; Qiu *et al.* 2007 and Yuan *et al.* 2007). Rice lines with more *Xa3* transcripts induced *OsWRKY13* and *NH1* more rapidly and/or effectively as compared with the rice lines with less *Xa3* transcripts or without carrying *Xa3*. These results suggest that rapid activation of OsWRKY13- and NH1-involved defense signal transduction might partly explain the enhanced resistance

associated with increased expression of *Xa3*.

Developmentally controlled resistance has been observed in many plant-pathogen systems. Full disease resistance usually occurs at adult stages in these systems. Rice *Xa21* is expressed at both susceptible and resistant stages, indicating that *Xa21*-mediated developmentally controlled disease resistance may not be related to its expression (Century *et al.* 1999). *Xa3* and *Xa21* encoding the same type of proteins share 53% sequence similarity (Sun *et al.* 2004). However, the present results indicate that the developmentally controlled *Xa3*-mediated resistance to some *Xoo* strains is associated with its expression level. This dosage-dependent developmental control may also be related to bacterial recognition specificity. Minghui 63 was highly resistant to *Xoo* strain JL691 at both seedling and adult stages (Chen *et al.* 2002), suggesting that XA3 can efficiently and specifically perceive the effector of JL691 and that more XA3 proteins are required for recognition of PXO61. *R* genes often express constitutively in either uninfected or infected plants (De Ilarduya and Kaloshian 2001; Shen *et al.* 2002; Paal *et al.* 2004; Huang *et al.* 2005; Schornack *et al.* 2005;), which agrees with their common role in pathogen recognition. This indicates that in most cases, the basal level of R proteins preexisting in cells is sufficient to guard pathogen invasion and initiate host resistance. However, in a few cases, pathogen induction increases *R* gene expression (Thurau *et al.* 2003; Levy *et al.* 2004; Gu *et al.* 2005). These results suggest that more R proteins are required on infection to help amplify the resistance response. *Xa3* belongs to the latter group of *R* genes. Low levels of pathogen-induced *Xa3* expression were constantly observed in *indica* rice lines and *japonica* transgenic lines in Mudanjiang 8 background. This result is consistent with the observation that increasing *Xa3* expression can enhance rice resistance. This induction was not detected in *japonica*

transgenic lines in Zhonghua 11 background, which may be due to very high levels of *Xa3* transcripts in these plants masking the light induction. However, a suppression of *Xa3* expression was also observed in only *indica* rice lines at early infection (4 h). Further study is needed to determine whether this is one of the causes of the impaired disease resistance in *indic*a lines as compared to *japonica* transgenic plants.

Xa3 preferentially expresses in the cells surrounding the vascular vessels (Y. Cao and S. Wang unpublished data), which perfectly fits the function of genes conferring resistance to *Xoo*, a vascular pathogen. Functional overlap between pathogen-induced defense signaling and plant development has been reported (Holt *et al.* 2002; Godiard *et al.* 2003; Chu *et al.* 2006), which may partly explain the fitness cost in disease resistance. Constitutive expression of an *R* gene sometimes results in plants with abnormal morphology or decreased fertility. Overexpression of the tomato *Pto* gene caused constitutive cell death (Tang *et al.* 1999). *Arabidopsis* overexpressing *RPW8* was lethal (Xiao *et al.* 2003). Even the native expression of *RPM1* influenced *Arabidopsis* development (Tian *et al.* 2003). Interestingly, *Xa3*-overexpressing plants showed no remarkable morphologic and developmental differences from wild type, which may contribute to, at least partly, the restricted expressional location of *Xa3*. Thus overexpression of *Xa3* can be applied to breeding programs to produce whole-growth-stage and wide-resistant-spectrum rice.

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TABLE 1

Resistance spectrum (lesion area in percent) of transgenic plants in *japonica* Zhonghua 11 background at booting stage

Xoo strain	Transgenic line		Zhonghua 11 (wild type)	*Xa3* donor Minghui 63	Transgenic line Rb17-2[a]
	MKbZH2[a]	MKbFZH2[a]			
PXO61	2.1 ± 0.6**	0.9 ± 0.3**	31.2 ± 4.1	14.4 ± 0.9	3.3 ± 1.7
PXO86	0.4 ± 0.1**	0.3 ± 0.0**	34.9 ± 4.3	9.1 ± 1.5	2.4 ± 1.2
PXO79	0.7 ± 0.3**	0.4 ± 0.1**	33.5 ± 8.5	10.5 ± 3.4	2.8 ± 1.0
PXO71	0.9 ± 0.3**	0.4 ± 0.2**	33.6 ± 10.9	57.8 ± 9.9	3.0 ± 1.1
PXO99	14.9 ± 1.9	10.9 ± 1.3**	16.2 ± 2.1	68.3 ± 4.9	83.8 ± 6.8
PXO145	0.6 ± 0.1**	0.3 ± 0.1**	24.3 ± 6.3	22.9 ± 3.9	2.6 ± 1.5
Z173	28.1 ± 4.3	26.5 ± 2.2**	35.6 ± 6.2	63.0 ± 7.4	81.7 ± 4.4

[a]MKbZH2, resistant T_1 plants carrying one copy of P_{Xa3}:*Xa3*; MKFZH2, resistant T_1 plants carrying one copy of P_{Ubi}:*Xa3*. Rb17-2 carries one copy of P_{Xa3}:*Xa3* with the background of *japonica* Mudanjiang 8.

**Significant difference ($P < 0.01$) was detected compared with wild type.

TABLE 2

Resistance spectrum (lesion area in percent) of transgenic plants in *japonica* Mudanjiang 8 and *indica* Minghui 63 backgrounds at booting stage

Xoo strain	Mudanjiang 8 background[a]			Minghui 63 background[a]	
	Rb17-2[b]	MKbFMDJ4, 5, 7[b]	Mudanjiang 8 (wild type)	MKbFMH2, 3, 4, 6, 7[b]	Minghui 63 (wild type)
PXO61	2.4 ± 0.8**	1.5 ± 0.7**	88.7 ± 10.2	8.0 ± 5.7**	46.1 ± 9.4
PXO79	1.5 ± 0.3**	0.8 ± 0.1**	89.6 ± 19.0	3.4 ± 1.5**	38.7 ± 8.2
PXO71	3.6 ± 1.8**	0.7**	81.9 ± 13.7	7.9 ± 4.9**	42.7 ± 9.6
PXO99	48.0 ± 16.9	54.3 ± 9.9	46.1 ± 6.2	59.0 ± 24.8	62.4 ± 14.0
PXO145	4.3 ± 0.9**	0.7 ± 0.1**	39.8 ± 8.8	9.3 ± 2.8**	35.4 ± 10.8
PXO280	12.0 ± 7.6**	1.2 ± 0.1**	59.5 ± 16.8		
T7174	18.5 ± 6.8	5.9 ± 0.6*	29.0 ± 14.7		
T7147	24.9 ± 10.2	3.5 ± 1.8**	21.4 ± 6.3		
T7133	2.2 ± 1.0**	1.1 ± 0.5**	85.7 ± 12.9	4.0 ± 1.0**	44.4 ± 19.1
Z173	25.4 ± 4.5	8.1 ± 6.6	29.1 ± 3.2	36.4 ± 6.2**	52.5 ± 6.8

[a]Plants with the two genetic backgrounds were inoculated with *Xoo* strains at different times.

[b]Rb17-2, homozygote transgenic line carrying one copy of P_{Xa3}:*Xa3*; MKbFMDJ4, 5, 7, resistant T_1 plants carrying one copy of P_{Ubi}:*Xa3*; MKbFMH2, 3, 4, 6, 7, resistant T_0 plants carrying P_{Ubi}:*Xa3*.

**Significant difference ($P < 0.01$) was detected compared with wild type.

*Significant difference ($P < 0.05$) was detected compared with wild type.

TABLE 3

Comparison of P_{Xa3}:*Xa3*- and P_{WRKY13}:*Xa3*-mediated resistance (lesion area in percent) to *Xoo* strain PXO61 at booting stage

	Zhonghua 11 background		Mudanjiang 8 background	
	MKbZH (P_{Xa3})	12IMKbZH (P_{WRKY13})	Rb17-2 (P_{Xa3})	12IMKbMDJ (P_{WRKY13})
T_0 plant[a]				
Range	0.4–3.3	7.7–25.7		36.2–53.1
Average	1.3 ± 1.0	14.2 ± 5.5**		41.5 ± 7.9
T_1 plant[a]				
Range	0.8–6.8	3.9–16.2	1.7–7.3	19.1–42.0
Average	2.5 ± 1.7	7.5 ± 3.2**	5.4 ± 0.9	22.9 ± 7.4**

[a]All positive transgenic plants are shown in Supplemental Table 2. Rb17-2 is the homozygote transgenic line.

**Significant difference ($P < 0.01$) from plants carrying P_{Xa3}:*Xa3* was detected.

TABLE 4

Reaction (lesion area in percent) of transgenic plants carrying P_{Xa3}:*Xa3* in different *japonica* backgrounds at seedling (four-leaf) stage

Xoo strain	*Indica* variety (*Xa3*)		Zhonghua 11 background		02428 background		Mudanjiang 8 background	
	Minghui 63	IRBB3	Transgenic[a]	Zhonghua 11 (wild type)	Transgenic[b]	02428 (wild type)	Transgenic[c]	Mudanjiang 8 (wild type)
PXO61	70.7 ± 4.4	75.4 ± 3.8	5.2 ± 1.3	59.2 ± 6.3	9.3 ± 1.1	100.0 ± 0.0	6.0 ± 1.5	100.0 ± 0.0
PXO71	76.1 ± 8.7	75.3 ± 6.7	6.6 ± 2.3	51.0 ± 7.4	10.3 ± 2.0	100.0 ± 0.0	5.4 ± 2.6	100.0 ± 0.0

[a]Resistant T_1 plants MKbZH2.

[b]Resistant T_1 plants MKb024-1.

[c]Resistant transgenic line Rb17-2.

Figure legends

Figure 1.—Performance of *Xa3* in different rice lines. Zhonghua 11, Mudanjiang 8, 02428, and Minghui 63 are wild types. Minghui 63 is also the donor of *Xa3*. The *Xa3* gene was driven by native promoter P_{Xa3} in plants named with prefix MKbZH, MKb024, or Rb; by P_{Ubi} in plants named MKbFZH, MKbFMDJ, or MKbFMH; and by P_{WRKY13} in plants 12MKbZH and 12IMKbMDJ. (A) Leaves from transgenic plants and wild types of booting stage at 14 days after inoculation with *Xoo* strain PXO61. Rb17-2 was a homozygote transgenic line. MKbFMDJ2 and 12IMKbMDJ7 were resistant T_1 plants, and other transgenic plants were T_0 generation. N, negative transgenic plants. (B) Growth of PXO61 in leaves of T_1 transgenic plants at booting stage. The bacterial population was determined from three leaves at each time point by counting colony-forming units (Sun *et al.* 2004). (C) Growth of PXO61 in leaves of T_1 transgenic plants at the four-leaf stage.

Figure 2.—Expression level of *Xa3* in different rice lines. Each RNA sample was from the mixture of at least three plants. (A) Genetic background influenced *Xa3* expression. Transgenic plants named with prefix MKbFMH, MKbFZH, MKbZH, 12IMKbZH, MKbFMDJ, 12IMKbMDJ, and MKb024-1 were resistant T_1 plants, and plants named with prefix Rb were homozygote transgenic lines. All the transgenic plants, except MKbFMH and MKb024-1, in which copy numbers were not determined, carried one copy of *Xa3*. *Xa3* was driven by the native promoter (P_{Xa3}), maize ubiquitin gene promoter (P_{Ubi}) or pathogen-induced *OsWRKY13* gene promoter (P_{WRKY13}) in the transgenic plants. (B) Developmental stage influenced *Xa3* expression. The expression level of *Xa3* in each developmental stage of each rice line was relative to that in the

two-leaf stage of Minghui 63.

Figure 3.—Expression levels of *OsWRKY13* and *NH1* in different rice lines. Plants were inoculated with *Xoo* strain PXO61 at booting stage. ck, without inoculation. The expression level of the genes in each time point of each rice line was relative to that in the ck of Minghui 63.

Figure 4.—*Xa3* expression on pathogen infection analyzed by RT-qPCR. Plants were inoculated with *Xoo* strain PXO61 at booting stage. ck, without inoculation; ckM, transgenic plant MKbZH2 without inoculation.

Figure 1

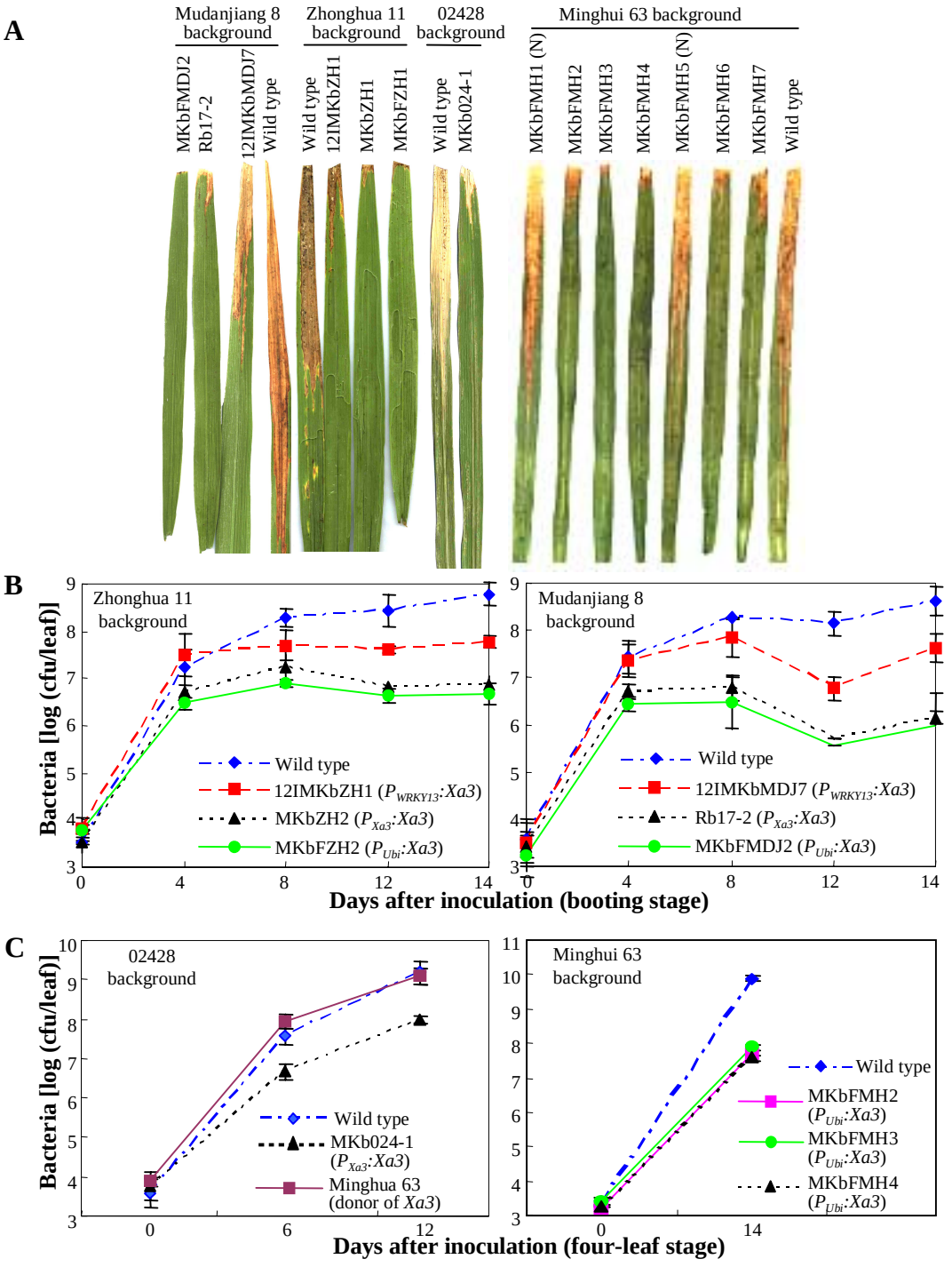
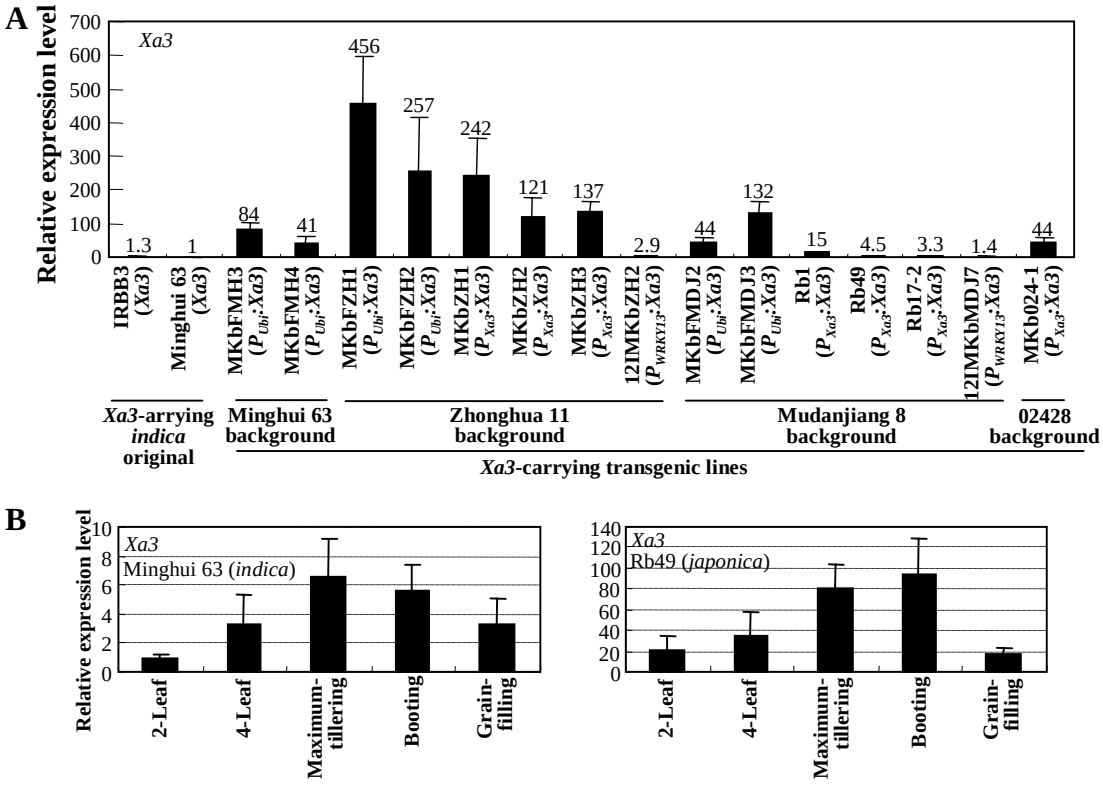

A
Mudanjiang 8 background
Zhonghua 11 background
02428 background
Minghui 63 background
MKbFMDJ2
Rb17-2
12IMKbMDJ7
Wild type
Wild type
12IMKbZH1
MKbZH1
MKbFZH1
Wild type
MKb024-1
MKbFMH1 (N)
MKbFMH2
MKbFMH3
MKbFMH4
MKbFMH5 (N)
MKbFMH6
MKbFMH7
Wild type
B
Zhonghua 11 background
Mudanjiang 8 background
Bacteria [log (cfu/leaf)]
Wild type
12IMKbZH1 (P_{WRKY13}:Xa3)
MKbZH2 (P_{Xa3}:Xa3)
MKbFZH2 (P_{Ubi}:Xa3)
12IMKbMDJ7 (P_{WRKY13}:Xa3)
Rb17-2 (P_{Xa3}:Xa3)
MKbFMDJ2 (P_{Ubi}:Xa3)
Days after inoculation (booting stage)
C
02428 background
Minghui 63 background
Bacteria [log (cfu/leaf)]
Wild type
MKb024-1 (P_{Xa3}:Xa3)
Minghua 63 (donor of Xa3)
MKbFMH2 (P_{Ubi}:Xa3)
MKbFMH3 (P_{Ubi}:Xa3)
MKbFMH4 (P_{Ubi}:Xa3)
Days after inoculation (four-leaf stage)

Figure 2


A
Relative expression level
Xa3
700
600
500
400
300
200
100
0
1.3
1
84
41
456
257
242
121
137
2.9
44
132
15
4.5
3.3
1.4
44
IRBB3 (Xa3)
Minghui 63 (Xa3)
MKbFMH3 (PUbi:Xa3)
MKbFMH4 (PUbi:Xa3)
MKbFZH1 (PUbi:Xa3)
MKbFZH2 (PUbi:Xa3)
MKbZH1 (PXa3:Xa3)
MKbZH2 (PXa3:Xa3)
MKbZH3 (PXa3:Xa3)
12IMKbZH2 (PWRKY13:Xa3)
MKbFMDJ2 (PUbi:Xa3)
MKbFMDJ3 (PUbi:Xa3)
Rb1 (PXa3:Xa3)
Rb49 (PXa3:Xa3)
Rb17-2 (PXa3:Xa3)
12IMKbMDJ7 (PWRKY13:Xa3)
MKb024-1 (PXa3:Xa3)
Xa3-arrying indica original
Minghui 63 background
Zhonghua 11 background
Mudanjiang 8 background
02428 background
Xa3-carrying transgenic lines

B
Relative expression level
Xa3
Minghui 63 (indica)
10
8
6
4
2
0
2-Leaf
4-Leaf
Maximum-tillering
Booting
Grain-filling
Xa3
Rb49 (japonica)
140
120
100
80
60
40
20
0
2-Leaf
4-Leaf
Maximum-tillering
Booting
Grain-filling

Figure 3

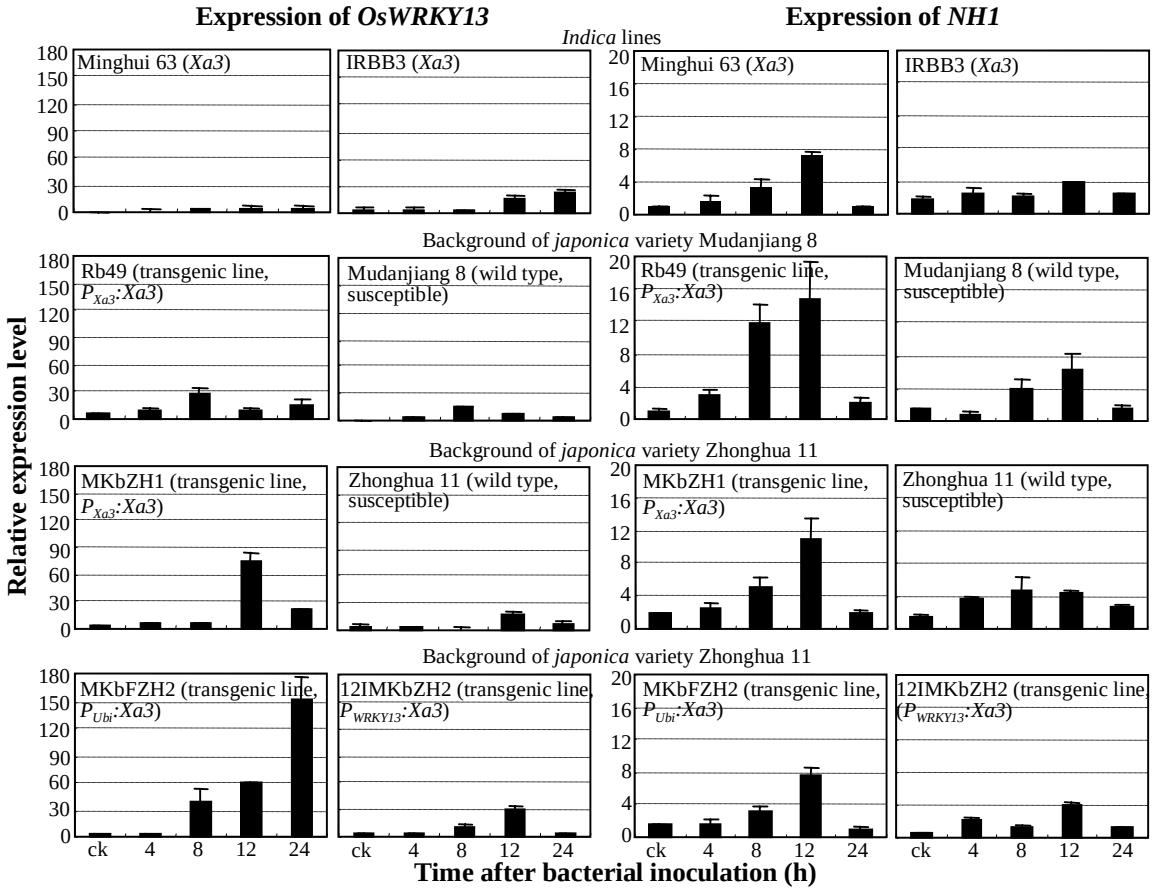
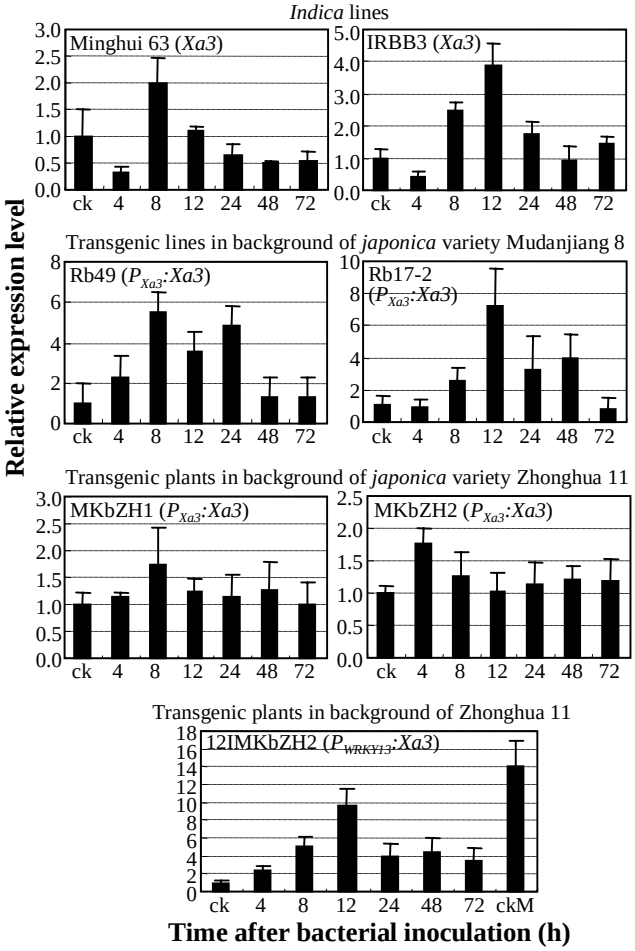

Expression of OsWRKY13
Expression of NH1
Indica lines
Minghui 63 (Xa3)
IRBB3 (Xa3)
Background of japonica variety Mudanjiang 8
Rb49 (transgenic line, P_Xa3:Xa3)
Mudanjiang 8 (wild type, susceptible)
Background of japonica variety Zhonghua 11
MKbZH1 (transgenic line, P_Xa3:Xa3)
Zhonghua 11 (wild type, susceptible)
Background of japonica variety Zhonghua 11
MKbFZH2 (transgenic line, P_Ubi:Xa3)
12IMKbZH2 (transgenic line, P_WRKY13:Xa3)
Relative expression level
ck 4 8 12 24
Time after bacterial inoculation (h)

Figure 4


Indica lines
Minghui 63 (*Xa3*)
IRBB3 (*Xa3*)
Transgenic lines in background of *japonica* variety Mudanjiang 8
Rb49 (P_{Xa3}:*Xa3*)
Rb17-2 (P_{Xa3}:*Xa3*)
Transgenic plants in background of *japonica* variety Zhonghua 11
MKbZH1 (P_{Xa3}:*Xa3*)
MKbZH2 (P_{Xa3}:*Xa3*)
Transgenic plants in background of Zhonghua 11
12IMKbZH2 (P_{WRKY13}:*Xa3*)
ck 4 8 12 24 48 72 ckM
Relative expression level
Time after bacterial inoculation (h)