A Pair of Allelic WRKY Genes Play Opposite Roles in Rice-Bacteria Interactions[1][C][W][OA]

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Although allelic diversity of genes has been reported to play important roles in different physiological processes, information on allelic diversity of defense-responsive genes in host-pathogen interactions is limited. Here, we report that a pair of allelic genes, *OsWRKY45-1* and *OsWRKY45-2*, which encode proteins with a 10-amino acid difference, play opposite roles in rice (*Oryza sativa*) resistance against bacterial pathogens. Bacterial blight caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*), bacterial streak caused by *Xanthomonas oryzae* pv *oryzicola* (*Xoc*), and fungal blast caused by *Magnaporthe grisea* are devastating diseases of rice worldwide. *OsWRKY45-1*-overexpressing plants showed increased susceptibility and *OsWRKY45-1*-knockout plants showed enhanced resistance to *Xoo* and *Xoc*. In contrast, *OsWRKY45-2*-overexpressing plants showed enhanced resistance and *OsWRKY45-2*-suppressing plants showed increased susceptibility to *Xoo* and *Xoc*. Interestingly, both *OsWRKY45-1*- and *OsWRKY45-2*-overexpressing plants showed enhanced resistance to *M. grisea*. OsWRKY45-1-regulated *Xoo* resistance was accompanied by increased accumulation of salicylic acid and jasmonic acid and induced expression of a subset of defense-responsive genes, while OsWRKY45-2-regulated *Xoo* resistance was accompanied by increased accumulation of jasmonic acid but not salicylic acid and induced expression of another subset of defense-responsive genes. These results suggest that both OsWRKY45-1 and OsWRKY45-2 are positive regulators in rice resistance against *M. grisea*, but the former is a negative regulator and the latter is a positive regulator in rice resistance against *Xoo* and *Xoc*. The opposite roles of the two allelic genes in rice-*Xoo* interaction appear to be due to their mediation of different defense signaling pathways.

Plant pathogens are continually evolving to survive. Plants have developed a set of mechanisms to face the challenge of foreign pathogens through a long history of coevolution. Among these mechanisms, maintaining allele (or ortholog) variation or diversity, either at the gene structure level or the expression level, is an important way for plants to protect themselves from pathogen attack. Plant responses to pathogen infection are regulated by different types of genes. The disease resistance (*R*) genes mediate race-specific resistance by initiation of defense signaling. The allelic variation of most characterized *R* genes and their alleles is regulated at the gene structure level; different resistant alleles of an *R* gene and its susceptible allele frequently encode different proteins (Sun et al., 2004; Zhou et al., 2006). In a few cases, the variation of *R* genes and their susceptible alleles is regulated expressionally (Gu et al., 2005; Chu et al., 2006; Romer et al., 2007). Based on our understanding of *R* gene-mediated resistance, dominant *R* genes function as positive regulators, and their susceptible alleles have no function in host-pathogen interaction (Gu et al., 2005; Romer et al., 2007); in contrast, recessive *R* genes appear to have no function, and their susceptible (dominant) alleles function as negative regulators in defense responses (Chu et al., 2006; Jiang et al., 2006). There is no report that an *R* gene and its allele function as positive and negative regulators in defense responses, respectively.

A large number of other genes, which function in the defense signaling pathways initiated by *R* genes or the pathways leading to basal immunity, respond to pathogen attack by changing expression levels or by post-translational modification of their encoding proteins. Thus, they are frequently called defense-responsive or defense-related genes. Although a large number of defense-responsive genes express differentially in host-pathogen interactions, the differential expression of most of these genes may be due to the activation of defense signaling in resistant reactions but not the variation of allelic expression, because gene expression was analyzed using near-isogenic lines for *R* genes (Zhou et al., 2002; Chu et al., 2004; Hulbert et al., 2007). Unlike the *R* genes, which are frequently subjected to positive selection, resulting in genetic diversity (Mondragon-Palomino et al., 2002; Sun et al., 2006), the defense-responsive genes have low levels

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of polymorphism and, in general, experience purifying selection (Bakker et al., 2008).

One important group of genes, which are also responsive to pathogen infection, is those encoding transcription factors that modulate the defense transcriptome. A number of WRKY-type transcription factors from different plant species have been identified to play important roles in host-pathogen interactions (Eulgem and Somssich, 2007; Pandey and Somssich, 2009). These WKRYs function either as positive or negative regulators in defense responses. Some WRKYs are both positive and negative regulators in different defense responses (Li et al., 2004, 2006; Wang et al., 2006; Xu et al., 2006). Some other WRKYs have partly redundant functions in defense signaling (Xu et al., 2006). However, there is no report that a WRKY gene or other type of defense-responsive gene and its allele function as a positive and negative regulators in defense responses, respectively.

Bacterial blight caused by *Xanthomonas oryzae* pv *oryzae* (*Xoo*), bacterial streak caused by *Xanthomonas oryzae* pv *oryzicola* (*Xoc*), and fungal blast caused by *Magnaporthe grisea* are devastating diseases of rice (*Oryza sativa*) worldwide. A numbers of *R* genes, but only a few resistance quantitative trait locus (QTL) genes for bacterial blight and blast resistance, have been isolated. A rice disease resistance QTL gene, *OsWRKY13*, which encodes a WRKY-type protein, is an important regulator of rice-*Xoo* and rice-*M. grisea* interactions (Qiu et al., 2007; Hu et al., 2008). Activation of OsWRKY13 can enhance rice resistance against *Xoo* and *M. grisea* (Qiu et al., 2007). In disease resistance, OsWRKY13's function is associated with activation of salicylic acid (SA)-dependent pathways and suppression of jasmonic acid (JA)-dependent pathways (Qiu et al., 2007, 2008). However, no *R* gene for *Xoc* resistance has been identified, and none of the resistance QTLs against *Xoc* has been characterized.

Our previous study showed that *OsWRKY45* (locus identifier LOC_Os05g25770), according to the rice genome annotation of The Institute for Genomic Research (http://rice.tigr.org), functioned downstream of OsWRKY13 (Qiu et al., 2009). Activation of *OsWRKY13* repressed *OsWRKY45* expression, and suppression of *OsWRKY13* enhanced *OsWRKY45* expression; furthermore, *Xoo* infection influenced *OsWRKY45* expression. These results suggest that *OsWRKY45* may be involved in rice-*Xoo* interactions. In addition, one study reported that OsWRKY45 transcription factor plays a crucial role in benzothiadiazole-inducible blast resistance (Shimono et al., 2007); another study showed that overexpressing *OsWRKY45* in Arabidopsis enhanced resistance to the bacterial pathogen *Pseudomonas syringae tomato* and enhanced tolerance to salt and drought stresses (Qiu and Yu, 2009). To study the role of *OsWRKY45* in rice response to *Xoo* infection, we found that the two alleles of *OsWRKY45* functioned differently in rice-pathogen interactions. We refer to the allele (Shimono et al., 2007) from *japonica* rice var Nipponbare as *OsWRKY45-1* and to the allele from *indica* rice var Minghui 63 as *OsWRKY45-2*. *OsWRKY45-1* acted as a negative regulator and *OsWRKY45-2* as a positive regulator in both rice-*Xoo* and rice-*Xoc* interactions, although both alleles functioned as positive regulators in rice-*M. grisea* interactions. The opposite roles of this pair of alleles in bacterial resistance appear to be due to their regulation of different defense signaling pathways.

RESULTS

Two Alleles of the *OsWRKY45* Gene in Different Rice Varieties

Asian cultivated rice consists of two major groups, which are known by the subspecies names *indica* and *japonica*. Amplification of *OsWRKY45* from *japonica* rice var Nipponbare and *indica* rice var Minghui 63 using gene-specific PCR primers generated differently sized PCR products (Fig. 1A). Comparative analysis of the genomic and cDNA sequences of *OsWRKY45* showed two homologous genes, defined as *OsWRKY45-1* (GenBank accession no. GQ331932) from Nipponbare and *OsWRKY45-2* (GQ331927) from Minghui 63 (Fig. 1B). The major differences between the two genes were a 502-nucleotide deletion in the first intron and a 12-nucleotide deletion in the third exon of *OsWRKY45-2* compared with *OsWRKY45-1*. *OsWRKY45-1* encodes a protein consisting of 326 amino acids, and *OsWRKY45-2* encodes a protein of 322 amino acids; in addition, the two proteins have six amino acid substitutions (Fig. 1C). *OsWRKY45* was also amplified from other rice varieties (Fig. 1A). Sequence comparison showed that another two *japonica* rice varieties, Mudanjiang 8 (GQ331930) and Dongjin (GQ331931), carried *OsWRKY45-1*, and another two *indica* rice varieties, Zhenshan 97 (GQ331928) and 93-11 (GQ331929), carried *OsWRKY45-2*.

To determine whether the two genes are in the same locus of each genome or if there are alleles, an allelic test was performed using an F2 population segregating for *OsWRKY45-1* and *OsWRKY45-2*. The numbers of F2 individuals carrying only *OsWRKY45-1*, both *OsWRKY45-1* and *OsWRKY45-2*, and only *OsWRKY45-2* were 42, 71, and 33, respectively, which fit the expected 1:2:1 ratio ($\chi^2 = 1.22$, $P > 0.5$). The two genes were mapped on chromosome 5 (Supplemental Fig. S1). In addition, BLAST analysis (Altschul et al., 1997) of the two gene sequences against Nipponbare and 93-11 whole genome sequences (http://rice.plantbiology.msu.edu/blast.shtml) identified only one gene with 100% coverage and 100% sequence identity to *OsWRKY45-1* in the Nipponbare genome and only one gene with 100% coverage and 99.8% sequence identity to *OsWRKY45-2* in the 93-11 genome. The homologs in Nipponbare and 93-11 genomes all localize on chromosome 5. These results suggest that *OsWRKY45-1* and *OsWRKY45-2* are alleles.

The promoter regions (approximately 1.5 kb upstream of the transcription initiation site) of different

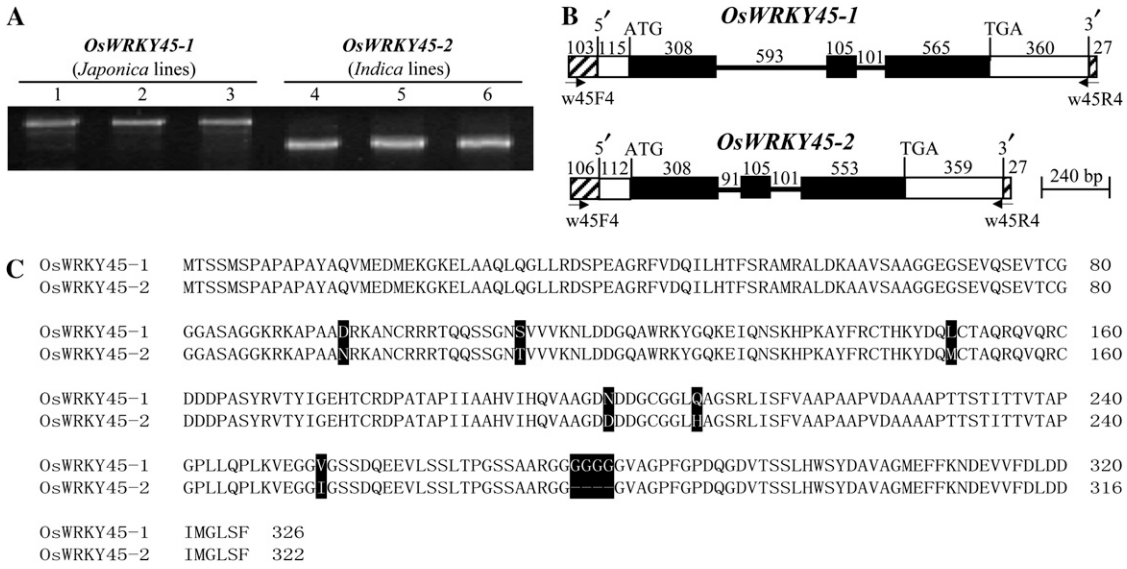

Figure 1. Structure and sequence comparison of *OsWKRY45-1* and *OsWRKY45-2*. A, Amplification of *OsWRKY45* using primers w45F4 and w45R4 showed differently sized PCR products in different rice varieties. The PCR products were 2,293 nucleotides in *japonica* var Nipponbare (1), Mudanjiang 8 (2), and Dongjin (3). The PCR products were 1,778 nucleotides for *indica* var Minghui 63 (4), Zhenshan 97 (5), and 93-11 (6). B, Gene structure. The coding regions (black boxes) of *OsWRKY45-1* and *OsWRKY45-2* are interrupted by introns (thick lines). The positions of 5′ and 3′ untranslated regions (white boxes), translation start codon (ATG), translation stop codon (TGA), and DNA fragments flanking the genes (hatched boxes) are also indicated. The numbers indicate the nucleotides of each substructure. C, Amino acid sequence alignment of OsWRKY45-1 and OsWRKY45-2 proteins. Dashes indicates a gap.

OsWRKY45 alleles from the six rice varieties were also compared. The *OsWRKY45-1* promoters from *japonica* var Nipponbare, Mudanjiang 8, and Dongjin had identical sequences. The *OsWRKY45-2* promoters from *indica* var Minghui 63, Zhenshan 97, and 93-11 also had identical sequences. There were 18 nucleotide substitutions and one insertion in the promoter region of *OsWRKY45-2* compared with that of *OsWRKY45-1* (Supplemental Fig. S2). These results suggest that there are at least two alleles of *OsWRKY45*, which appear to occur differentially in the two subspecies of Asian cultivated rice. Since *OsWRKY45-1* is involved in benzothiadiazole-inducible blast resistance (Shimono et al., 2007), these results also suggest that the two alleles may function differently in rice-pathogen interactions.

OsWRKY45-1 and *OsWRKY45-2* Play Opposite Roles in Rice-*Xoo* Interactions

To examine the above hypothesis, *OsWRKY45-1* and *OsWRKY45-2*, driven by a constitutive (maize [*Zea mays*] ubiquitin gene) promoter (P_{Ubi}), were transferred into *japonica* rice var Mudanjiang 8, which is susceptible to *Xoo* strain PXO61 (Qiu et al., 2007). An RNA interference construct for *OsWRKY45-2* was transferred into *indica* rice var Minghui 63, which is moderately resistant to PXO61 (Qiu et al., 2009). An *OsWRKY45-1*-knockout mutant, 2C-50229, which had the genetic background of *japonica* var Dongjin, was used for analysis.

Ten independent transformants carrying P_{Ubi}:*OsWRKY45-1*, named D113UM3 to D113UM12, were obtained. Four of the 10 T_0 plants carrying P_{Ubi}:*OsWRKY45-1* were significantly more susceptible ($P < 0.05$) to *Xoo* strain PXO61, with lesion areas ranging from 66% to 69%, compared with 55% for wild-type Mudanjiang 8 (Supplemental Fig. S3). To verify that the increased susceptibility of the transgenic plants was due to overexpression of *OsWRKY45-1*, two T_1 families from D113UM10 and D113UM11 were analyzed for susceptibility by inoculation with PXO61 and for *OsWRKY45-1* expression level. The results showed that all of the susceptible plants overexpressed *OsWRKY45-1* (Fig. 2A). The growth rate of bacteria on *OsWRKY45-1*-overexpressing plants was 2.5- to 43.8-fold higher than that on wild-type Mudanjiang 8 at 10 to 14 d after infection (Fig. 2B). T_2 plants from the D113UM11-33 line were further examined for their responses to different *Xoo* strains. The transgenic plants were significantly more susceptible ($P < 0.05$) to *Xoo* strains PXO86, PXO79, PXO99, Zhe173, and KS-1-21 than the wild type (Fig. 2C). These results suggest that overexpression of *OsWRKY45-1* resulted in a broad-spectrum susceptibility to *Xoo* strains.

The response of the *OsWRKY45-1*-knockout mutant (2C-50229), which had a T-DNA inserted into its promoter (Supplemental Fig. S4A), to *Xoo* strain PXO86 was examined. The plants with homozygote

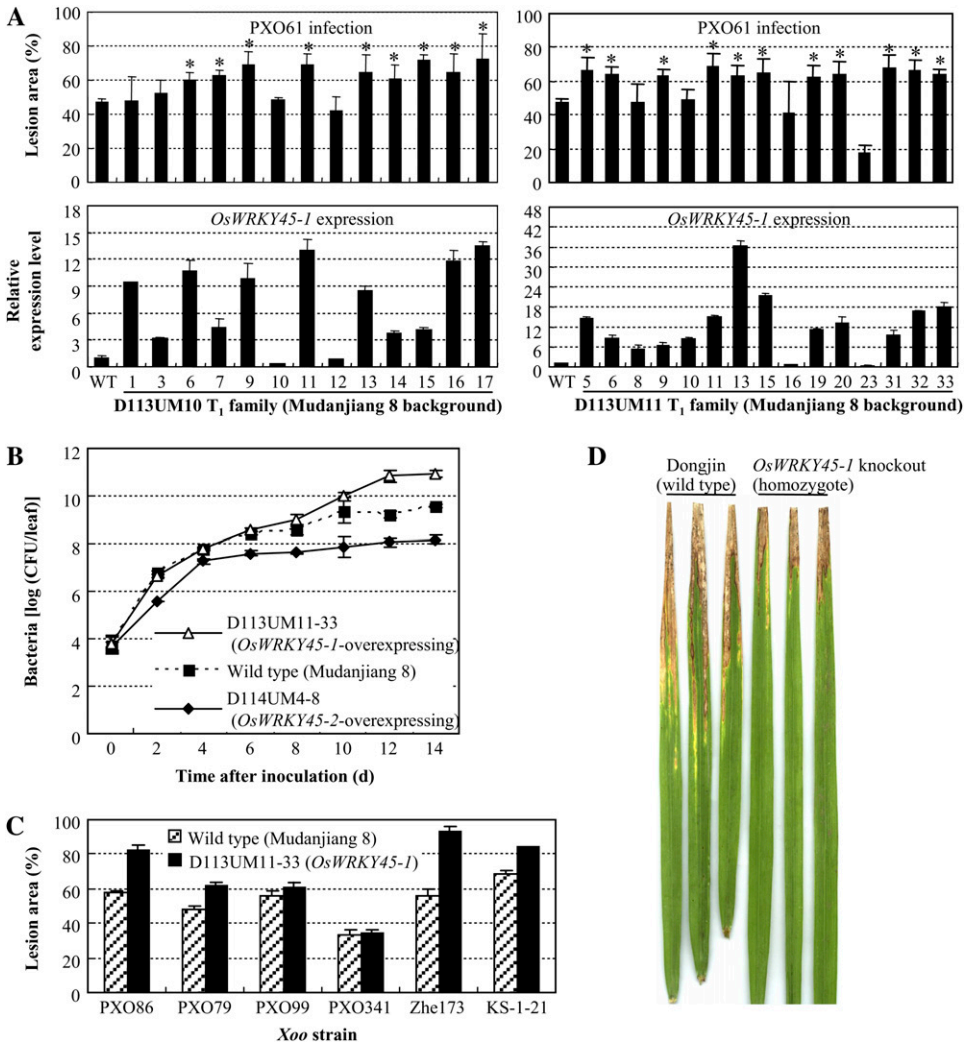

Figure 2. The role of *OsWRKY45-1* in rice-*Xoo* interaction. Bars represent means (four to five replicates for the lesion area and three replicates for expression level) ± SD. A, Increased susceptibility to *Xoo* strain PXO61 was associated with overexpression of *OsWRKY45-1* in *OsWRKY45-1*-overexpressing T_1 families D113UM10 and D113UM11. The asterisks indicate that a significant difference ($P < 0.05$) in the lesion area was detected between transgenic plants and the wild type (WT). The expression levels of *OsWRKY45-1* in transgenic plants are relative to that in the wild type. B, Growth of PXO61 in leaves of T_2 plants of D113UM11-33 overexpressing *OsWRKY45-1* and D114UM4-8 overexpressing *OsWRKY45-2*. Bacterial populations were determined from three leaves at each time point by counting colony-forming units (CFU). C, *OsWRKY45-1*-overexpressing transgenic line D113UM11-33 was also susceptible to other *Xoo* strains. D, Knockout of *OsWRKY45-1* (2C-50229) enhanced rice resistance to *Xoo* strain PXO86. [See online article for color version of this figure.]

T-DNA insertion, in which the expression level of *OsWRKY45-1* was only approximately 2% to 5% of that in wild-type Dongjin, as detected by quantitative reverse transcription (qRT)-PCR, showed markedly enhanced resistance, with an average lesion area of 20% compared with 46% for the wild type (Fig. 2D; Supplemental Fig. S4, B and C). These results suggest that reducing *OsWRKY45-1* transcripts can enhance rice resistance to *Xoo*. In conclusion, *OsWRKY45-1* acts as a negative regulator in the rice response to *Xoo* infection.

Seventeen independent transformants carrying P_{Ubi}:*OsWRKY45-2*, named D114UM1 to D114UM17, were obtained. Sixteen of the 17 T_0 plants carrying P_{Ubi}:*OsWRKY45-2* showed significantly enhanced resistance to *Xoo* strain PXO61, with lesion areas ranging from 5% to 28% compared with 55% for wild-type Mudanjiang 8 (Supplemental Fig. S5). Two T_1 families from resistant D114UM4 and D114UM6 were further analyzed for resistance to PXO61 and for *OsWRKY45-2* expression level. The results showed that the enhanced resistance was associated with overexpression of *OsWRKY45-2* in the T_1 families (Fig. 3A). The bacterial growth rate in *OsWRKY45-2*-overexpressing plants was 2- to 20-fold lower than that in the wild type at 2 to 14 d after infection (Fig. 2B). T_2 plants from the D114UM4-8 line were further examined for their resistance spectrum to different *Xoo* strains. The transgenic plants showed markedly enhanced resistance to *Xoo* strains PXO86, PXO79, PXO99, PXO341, Zhe173, and KS-1-21 compared with the wild type (Fig. 3B). The lesion areas of the transgenic plants were reduced 51% to 94% compared with wild-type Mudanjiang 8. These results suggest that an increasing expression level of *OsWRKY45-2* can promote a broad-spectrum resistance to *Xoo* strains.

Thirty-one independent transformants carrying the *OsWRKY45-2* RNA interference construct, named D115RMH1 to D115RMH31, were obtained. Minghui 63 is moderately resistant to *Xoo* strain PXO61 (Sun et al., 2004). Some of the T_0 plants showed decreased resistance to PXO61 compared with wild-type Minghui 63 (data not shown). Three T_1 families developed from three susceptible T_0 plants, D115RMH1, -6, and

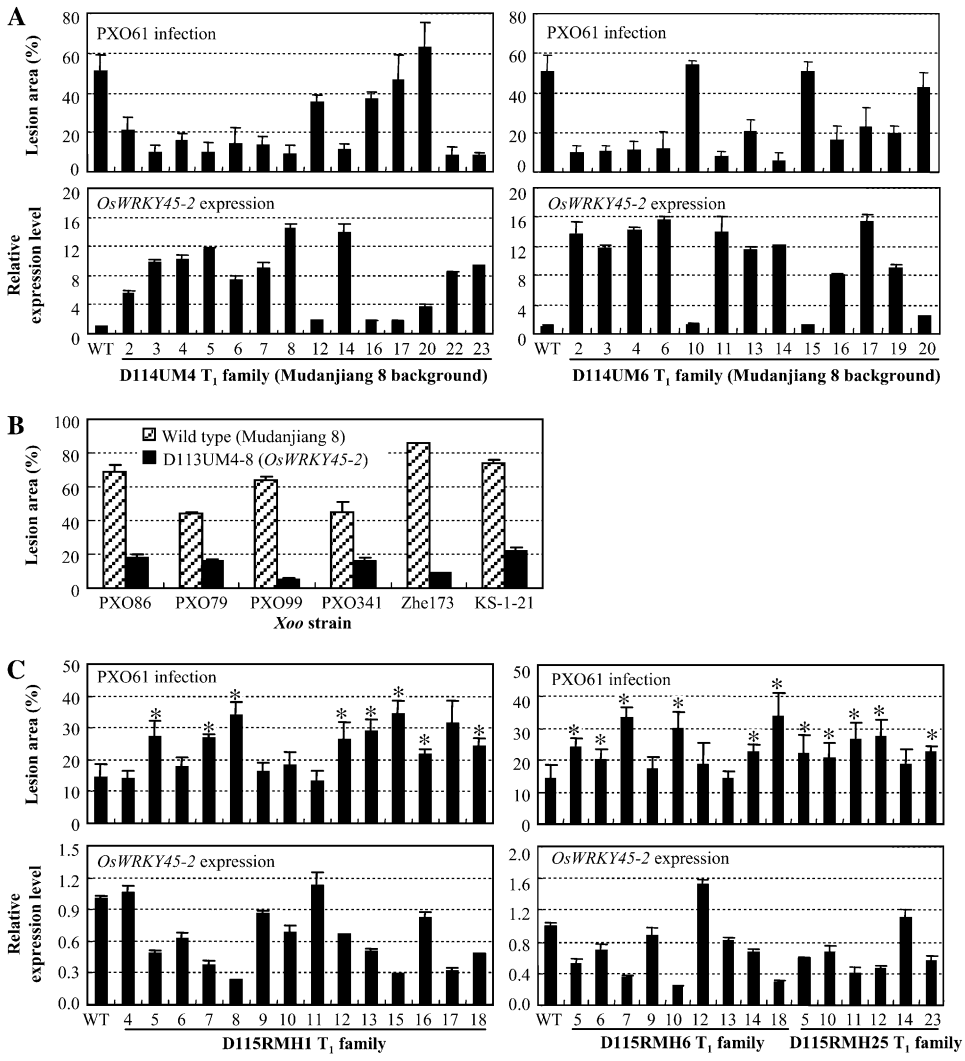

Figure 3. The role of *OsWRKY45-2* in rice-*Xoo* interaction. The asterisks indicate that a significant difference ($P < 0.05$) in the lesion area was detected between transgenic plants and the wild type (WT). The expression levels of *OsWRKY45-2* in transgenic plants are relative to that in the wild type. Bars represent means (four to five replicates for the lesion area and three replicates for expression level) ± SD. A, Enhanced resistance to *Xoo* strain PXO61 associated with overexpression of *OsWRKY45-2* in T_1 families D114UM4 and D114UM6. B, The *OsWRKY45-2*-overexpressing line D113UM4-8 was also resistant to other *Xoo* strains. C, Suppressing *OsWRKY45-2* decreased rice resistance to *Xoo* strain PXO61. Minghui 63 is the wild type.

-25, were further analyzed for their response to PXO61 and the *OsWRKY45-2* transcript level. The results showed that the decreased expression levels of *OsWRKY45-2* were associated with increased susceptibility in all of the T_1 families (Fig. 3C). These results suggest that *OsWRKY45-2* acts as a positive regulator in rice response to *Xoo* infection.

The Two Alleles Have Different Expression Responses to *Xoo* Infection

To evaluate whether the opposite functions of the two alleles in rice-*Xoo* interactions were due to their different expression patterns in pathogen infection, we examined their expression in different rice lines (Fig. 4). Rice var Mudanjiang 8, which is susceptible to *Xoo* strain PXO61, carries *OsWRKY45-1*. Transgenic line Rb49 carries an *R* gene, *Xa3/Xa26*, against PXO61 and has the genetic background of Mudanjiang 8 (Cao et al., 2007). Rice var Minghui 63 and Zhenshan 97 carry *OsWRKY45-2*, and the former, carrying *Xa3/Xa26*, is moderately resistant to PXO61, whereas the latter is susceptible to PXO61 (Cao et al., 2007). Although PXO61 infection induced *OsWRKY45-1* and *OsWRKY45-2* expression in both resistant and susceptible rice lines, the two alleles showed different expression patterns in resistant lines as compared with corresponding susceptible lines (Fig. 4). The expression level of *OsWRKY45-1* in the resistant line was higher than that in the susceptible line without pathogen infection but was markedly lower than that in the susceptible line in early infection (1 h). In contrast, the expression of *OsWRKY45-2* in the resistant line was markedly induced at 12 h after infection. Because *OsWRKY45-1* and its promoter are identical in Mudanjiang 8 and Rb49 and *OsWRKY45-2* and its promoter are identical in Zhenshan 97 and Minghui 63, the expression differences of the two alleles in susceptible and resistant lines may be due to the differential regulation by other transcriptional regulator(s) that is activated or suppressed during rice-*Xoo* interaction. Furthermore, the different expression patterns of *OsWRKY45-1* and *OsWRKY45-2* in resistant lines, as compared with those in their corresponding suscepti-

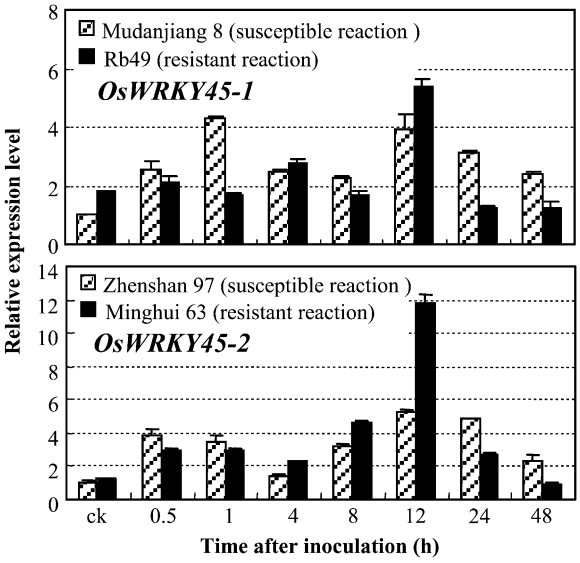

Figure 4. *OsWRKY45-1* and *OsWRKY45-2* expression on pathogen infection. Plants were inoculated with *Xoo* strain PXO61 at the booting stage. ck, Before inoculation. Bars represent means (three replicates) ± SD.

ble lines, suggest that the two alleles may be regulated by different transcriptional regulators due to promoter difference.

OsWRKY13 Transcription Regulator Binds to the Promoters of *OsWRKY45-1* and *OsWRKY45-2* in Vivo

OsWRKY13 bound to the promoter of *OsWRKY45* in vitro (Qiu et al., 2009). To evaluate whether OsWRKY13 was the direct regulator of *OsWRKY45*, we performed chromatin immunoprecipitation assay using anti-OsWRKY13 antibody (Supplemental Fig. S6). The samples were from rice var Mudanjiang 8, carrying *OsWRKY45-1*, and Minghui 63, carrying *OsWRKY45-2*. Three segments of *OsWRKY45-1* and *OsWRKY45-2* promoters, which putatively harbor W-boxes or W-box-like cis-elements for the binding of WRKY transcription factors or polymorphic sites, were analyzed (Supplemental Fig. S2). After immunoprecipitation with anti-OsWRKY13 antibody, enrichment of the first (P45F3/P45R3) and second (P45F2/P45R2) fragments of the *OsWRKY45-1* promoter and the third (P45F4/P45R4) fragment of the *OsWRKY45-2* promoter was detected by real-time PCR (Fig. 5). This result suggests that OsWRKY13 binds to the promoters of *OsWRKY45-1* and *OsWRKY45-2* in vivo, but it has partiality to bind to the different sites of the two promoters.

OsWRKY45-1 and *OsWRKY45-2* Differentially Regulate the Expression of a Set of Defense-Responsive Genes

To ascertain which defense-responsive genes were influenced by OsWRKY45-1 or OsWRKY45-2, we analyzed the expression of 10 genes known to function in SA- or JA-dependent pathways in different rice plants after infection of *Xoo* strain PXO61 (Fig. 6). *PAL1* (for Phe ammonia lyase 1; GenBank accession no. X16099) is involved in SA synthesis by the phenylpropanoid pathway. *ICS1* (for isochorismate synthase 1; AK120689) and *PAD4* (for phytoalexin-deficient 4; CX118864) are putatively involved in SA synthesis in rice by the isochorismate pathway (Qiu et al., 2007). Induced expression of *PR1a* (for acidic pathogenesis-related [PR] protein 1; AJ278436), *NH1* (for Arabidopsis [*Arabidopsis thaliana*] *NPR1* homolog 1; AY9123983), or *OsWRKY13* (EF143611) was associated with activation of the SA-dependent pathway. *LOX* (for lipoxygenase; D14000) and *AOS2* (for allene oxide synthase 2; AY062258) are involved in JA synthesis. *PR1b* (for basic PR protein 1; U89895) and *PR10/PBZ1* (for ribonuclease; D38170) appear to function in both JA- and SA-dependent pathways (Qiu et al., 2007; Yuan et al., 2007; X. Shen and S. Wang, unpublished data).

In *OsWRKY45-1*-containing plants, the expression of *PAL1*, *PAD4*, *PR1a*, *NH1*, *LOX*, and *PR1b* was significantly increased ($P < 0.01$) in *OsWRKY45-1*-knockout plants (enhanced resistance) compared with wild-type Dongjin and was significantly suppressed ($P < 0.01$) in *OsWRKY45-1*-overexpressing plants (increased susceptibility) compared with wild-type Mudanjiang 8 in at least one time point examined (Fig. 6A). Both *OsWRKY45-1*-knockout and -overexpressing plants showed suppressed expression of *AOS2*, *PR10/PBZ1*, and *OsWRKY13* and slightly increased expression of

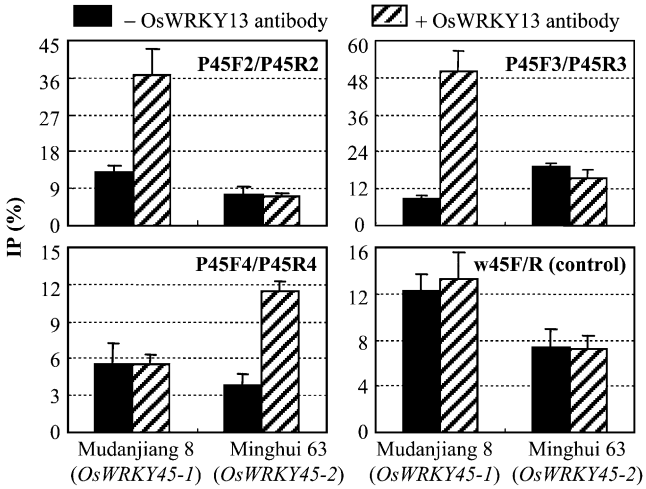

Figure 5. OsWRKY13 protein binds to the promoters of *OsWRKY45-1* and *OsWRKY45-2*. Chromatin immunoprecipitation assay was performed with the extracts from the leaves of Mudanjiang 8 and Minghui 63 seedlings. Real-time PCR was conducted before immunoprecipitation (input), after immunoprecipitation (IP) with anti-OsWRKY13 antibody (+), or after immunoprecipitation without anti-OsWRKY13 antibody (−). The amplification of the 3′ untranslated region of *OsWRKY45-1* or *OsWRKY45-2* using PCR primer pair w45F/R served as a sample quantity control. The amounts of target PCR products from IP are relative (IP/input %) to those from input. Bars represent means (three replicates) ± SD.

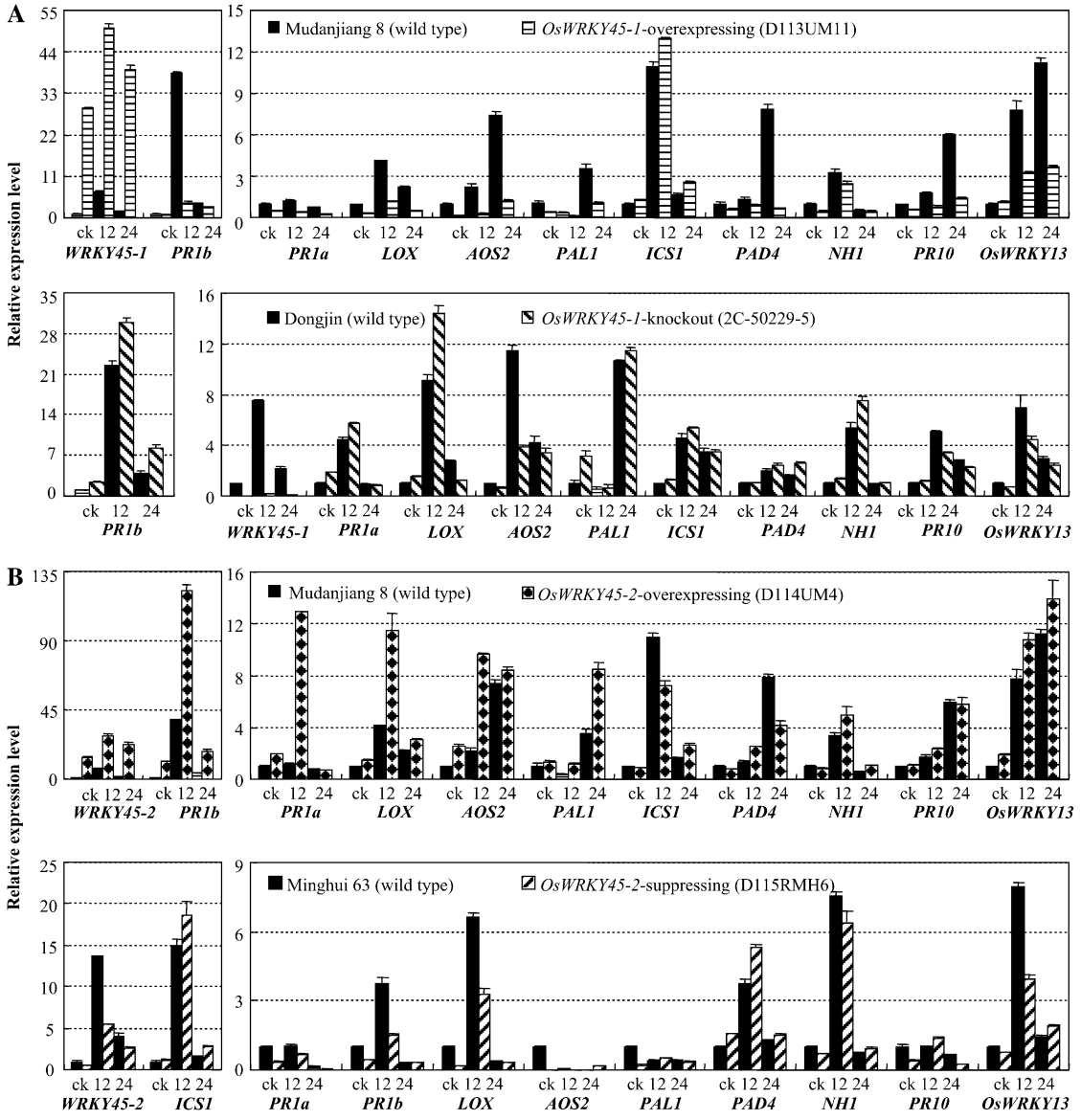

Figure 6. Modulating *OsWRKY45-1* (A) and *OsWRKY45-2* (B) expression influenced the expression of other defense-responsive genes analyzed by qRT-PCR. *AOS2*, Allene oxide synthase 2; *ICS1*, isochorismate synthase 1; *LOX*, lipoxygenase; *NH1*, *NPR1* homolog 1; *PAD4*, phytoalexin-deficient 4; *PAL1*, Phe ammonia lyase 1; *PR1a*, acidic pathogenesis-related protein 1; *PR1b*, basic pathogenesis-related protein 1; *PR10/PBZ1*, ribonuclease. Samples were collected before inoculation (ck) and at 12 and 24 h after inoculation with *Xoo* strain PXO61. Bars represent means (three replicates) ± SD.

ICS1. These results suggest that OsWRKY45-1 may play an important role in regulating the expression of *PAL1*, *PAD4*, *PR1a*, *NH1*, *LOX*, and *PR1b*.

In *OsWRKY45-2*-containing plants, the expression of *PAL1*, *PR1a*, *NH1*, *OsWRKY13*, *LOX*, *AOS2*, and *PR1b* was significantly increased ($P < 0.01$) in *OsWRKY45-2*-overexpressing plants (enhanced resistance) compared with wild-type Mudanjiang 8 and was significantly suppressed ($P < 0.01$; *NH1*, $P < 0.05$) in *OsWRKY45-2*-suppressing plants (increased susceptibility) compared with wild-type Minghui 63 in at least one time point examined (Fig. 6B). The expression of *ICS1* and *PAD4* was suppressed in *OsWRKY45-2*-overexpressing plants and induced in *OsWRKY45-2*-suppressing plants compared with their corresponding wild-type plants in at least one time point examined. *PR10/PBZ1* expression showed no obvious difference between *OsWRKY45-2*-overexpressing and wild-type plants but was significantly suppressed ($P < 0.01$) in *OsWRKY45-2*-suppressing plants compared with wild-type Minghui 63 (Fig. 6B). These results suggest that OsWRKY45-2 may play an important role in regulating the expression of *PAL1*, *ICS1*, *PAD4*, *PR1a*, *NH1*, *OsWRKY13*, *LOX*, *AOS2*, and *PR1b*. The

different expression patterns of this set of defense-responsive genes in *OsWRKY45-1*- and *OsWRKY45-2*-containing plants suggest that this pair of alleles may regulate rice-*Xoo* interactions by different defense signaling.

OsWRKY45-1 and *OsWRKY45-2* Influence the Levels of Endogenous SA and JA

To examine whether the modified expression of defense-responsive genes caused by *OsWRKY45* influences the endogenous levels of JA and SA, we quantified the concentrations of the two signal molecules in the leaves of the same plants used for analyzing the expression of defense-responsive genes after infection of *Xoo* strain PXO61. In *OsWRKY45-1*-containing wild-type plants, PXO61 infection markedly induced JA accumulation in both Mudanjiang 8 and Dongjin but only slightly induced SA accumulation in Mudanjiang 8 (Fig. 7). In *OsWRKY45-2*-containing wild-type Minghui 63, PXO61 infection induced JA accumulation and suppressed SA accumulation. The SA and JA levels were significantly reduced ($P < 0.05$) in *OsWRKY45-1*-overexpressing plants and were significantly increased ($P < 0.05$) in *OsWRKY45-1*-knockout plants compared with their corresponding wild types (Fig. 7). The JA level was significantly increased ($P < 0.05$) in *OsWRKY45-2*-overexpressing plants and decreased in *OsWRKY45-2*-suppressing plants compared with their corresponding wild types. The SA levels in both *OsWRKY45-2*-overexpressing and -suppressing plants showed no significant differences ($P > 0.05$) from their corresponding wild types. In conclusion, the *Xoo* resistance mediated by *OsWRKY45-1*-knockout plants was associated with increased accumulation of SA and JA, and the *Xoo* resistance mediated by *OsWRKY45-2*-overexpressing plants was associated with accumulation of JA but not SA. These results further support the hypothesis that the *Xoo* resistance negatively regulated by OsWRKY45-1 is different from that positively regulated by OsWRKY45-2.

OsWRKY45-1 and *OsWRKY45-2* Also Play Opposite Roles in Rice-*Xoc* Interactions

The *OsWRKY45-1*-overexpressing lines (D113UM10 and D113UM11) were more susceptible to *Xoc* strain RH3; the lesion lengths of these transgenic plants were increased 73% to 95% compared with wild-type Mudanjiang 8, which was moderately susceptible to *Xoc* (Table I). In contrast, the *OsWRKY45-1*-knockout plants (2C-50229-5) were more resistant to RH3; the lesion lengths of these plants were reduced 47% compared with wild-type Dongjin, which was moderately susceptible to *Xoc*. The *OsWRKY45-2*-overexpressing lines (U114UM4 and D114UM6) were more resistant to

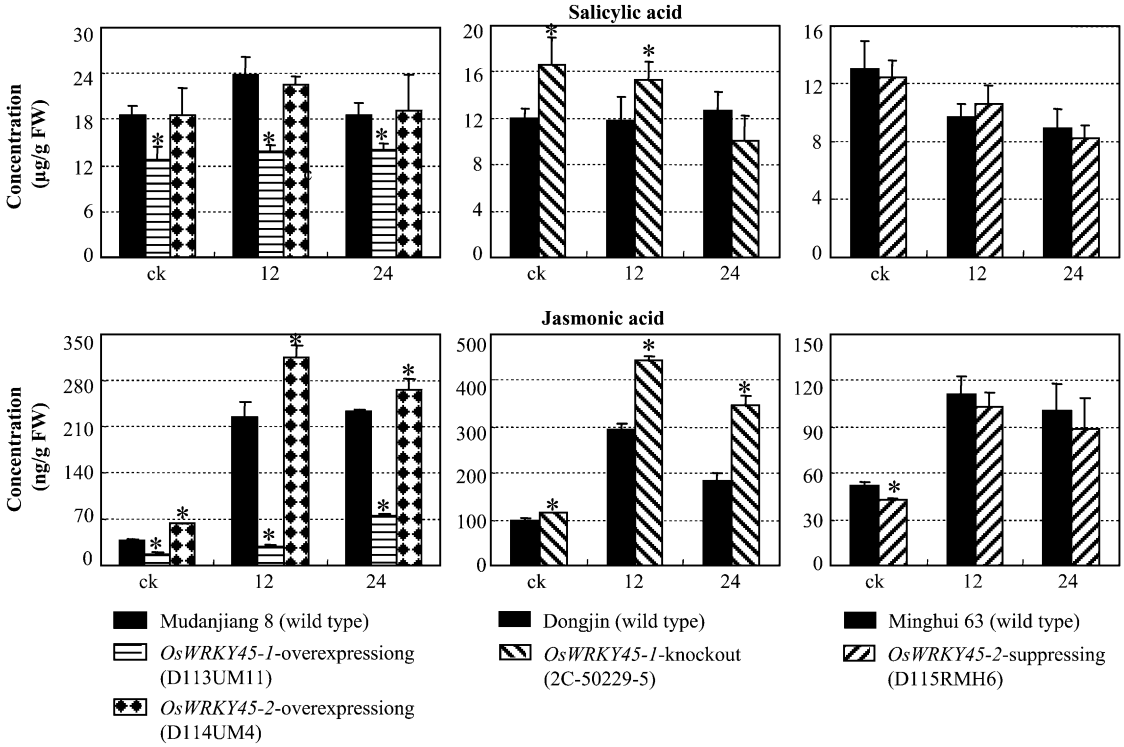

Figure 7. Modulating *OsWRKY45-1* and *OsWRKY45-2* expression influenced the accumulation of SA and JA. Samples were collected before inoculation (ck) and at 12 and 24 h after inoculation with *Xoo* strain PXO61. Bars represent means (three replicates) ± SD. Asterisks indicate that a significant difference ($P < 0.05$) was detected between transgenic plants and the corresponding wild type of the same treatment. FW, Fresh weight.

RH3 compared with wild-type Mudanjiang 8; the lesion lengths of these transgenic plants were reduced 52% to 56% (Table I). In contrast, the *OsWRKY45-2*-suppressing lines (D115RMH1 and D115RMH6) were more susceptible to *Xoc*; the lesion lengths of these transgenic plants were increased approximately 17% compared with wild-type Minghui 63, which was susceptible to *Xoc*. These results suggest that OsWRKY45-1 negatively regulates rice resistance to *Xoc* and that OsWRKY45-2 positively regulates rice resistance to *Xoc*.

Both *OsWRKY45-1* and *OsWRKY45-2* Positively Regulate Rice Resistance against *M. grisea*

A previous study reported that overexpression of *OsWRKY45* (named *OsWRKY45-1* in this study) enhanced rice resistance to blast disease (Shimono et al., 2007). To ascertain whether this pair of alleles also had different responses in rice-*M. grisea* interactions, we inoculated the transgenic plants with *M. grisea* isolate 91-17-2. Both *OsWRKY45-1*- and *OsWRKY45-2*-overexpressing plants showed enhanced resistance to *M. grisea* compared with wild-type Mudanjing 8, which was highly susceptible (Table II). However, the *OsWRKY45-2*-overexpressing plants were more resistant to 91-17-2 than *OsWRKY45-1*-overexpressing plants. In contrast, *OsWRKY45-1*-knockout and *OsWRKY45-2*-suppressing plants were more susceptible to 91-17-1 compared with wild-type Dongjin, which was resistant, and Minghui 63, which was moderately susceptible, respectively (Table II). These results suggest that both OsWRKY45-1 and OsWRKY45-2 act as positive regulators in rice-*M. grisea* interactions.

DISCUSSION

OsWRKY45-1 and OsWRKY45-2 Modulate Rice-*Xoo* Interactions via Different Mechanisms

The WRKY superfamily of rice consists of at least 98 members in *japonica* rice and 102 members in *indica* rice (Ross et al., 2007). Nine rice WRKY genes have been characterized to be involved in pathogen-induced defense responses. OsWRKY13 positively regulates rice defense responses against both *Xoo* and *M. grisea* (Qiu et al., 2007). OsWRKY71 positively regulates rice resistance to *Xoo* (Liu et al., 2007). OsWRKY53, OsWRKY45-1, OsWRKY89, and OsWRKY31 are positive regulators of rice resistance to *M. grisea* (Chujo et al., 2007; Shimono et al., 2007; Wang et al., 2007; Zhang et al., 2008). Although 15 Arabidopsis WRKY proteins have been identified to negatively regulate defense responses against different pathogens (Pandey and Somssich, 2009), only one rice WRKY protein, OsWRKY62, has been reported to be a negative regulator in rice pathogen defense (Peng et al., 2008). OsWRKY62 negatively regulates defense responses in basal and *Xa21*-mediated resistance against *Xoo*. One WRKY protein from pepper (*Capsicum annuum*) also functions as a negative regulator of pathogen defense (Oh et al., 2008). In adding to this list, our results suggest that both OsWRKY45-1 and OsWRKY45-2 are positive regulators in rice resistance against *M. grisea*, but the former is a negative regulator and the latter is a positive regulator in rice resistance against *Xoo* and *Xoc*.

The opposite functions of OsWRKY45-1 and OsWRKY45-2 in rice-*Xoo* interactions are controlled by different defense signaling pathways. This hypothesis is supported by the following evidence. First, the *Xoo* resistance, which was negatively regulated by OsWRKY45-1, was associated with increased accumulation of SA and JA, but the *Xoo* resistance, which was positively regulated by OsWRKY45-2, was only associated with increased accumulation of JA. Second, the expression patterns of a subset of defense-responsive genes were different in the OsWRKY45-1- and OsWRKY45-2-mediated disease resistance. The expression of *ICS1* and *PAD4*, which are putatively involved in SA biosynthesis in rice via the isochorismate pathway (Qiu et al., 2007), was increased in OsWRKY45-1 negatively regulated resistance and appeared to be suppressed in OsWRKY45-2 positively regulated resistance. OsWRKY45-1-mediated resistance was accompanied by suppressed expression of *OsWRKY13* and slightly induced expression of *PR1a* and *PR1b*, but OsWRKY45-2-mediated resis-

Table I. *Performance of transgenic plants inoculated with Xoc strain RH3*

Rice Line	Genetic Background	Lesion Length[a]	*P*
		cm	
D113UM10 (*OsWRKY45-1*-overexpressing)	Mudanjiang 8	2.58 ± 0.07	0.0000
D113UM11 (*OsWRKY45-1*-overexpressing)	Mudanjiang 8	2.29 ± 0.19	0.0019
D114UM4 (*OsWRKY45-2*-overexpressing)	Mudanjiang 8	0.58 ± 0.04	0.0004
D114UM6 (*OsWRKY45-2*-overexpressing)	Mudanjiang 8	0.63 ± 0.06	0.0001
Mudanjiang 8 (wild type)	Mudanjiang 8	1.32 ± 0.08	
2C-50229-5 (*OsWRKY45-1*-knockout)	Dongjin	0.73 ± 0.11	0.0009
Dongjin (wild type)	Dongjin	1.38 ± 0.11	
D115RMH1 (*OsWRKY45-2*-suppressing)	Minghui 63	2.37 ± 0.10	0.0084
D115RMH6 (*OsWRKY45-2*-suppressing)	Minghui 63	2.39 ± 0.18	0.0405
Minghui 63 (wild type)	Minghui 63	2.03 ± 0.06	

[a]Data represents means (three plants each with four lesions per plant) ± SD.

Table II. *Performance of transgenic plants inoculated with M. grisea isolate 91-17-2*

Rice Line	Genetic Background	Disease Index[a]	Resistance/Susceptibility
D113UM11 (*OsWRKY45-1*-overexpressing)	Mudanjiang 8	38.4	Moderately susceptible
D113UM10 (*OsWRKY45-1*-overexpressing)	Mudanjiang 8	39.3	Moderately susceptible
D114UM4 (*OsWRKY45-2*-overexpressing)	Mudanjiang 8	17.9	Moderately resistant
D114UM6 (*OsWRKY45-2*-overexpressing)	Mudanjiang 8	13.4	Resistant
Mudanjiang 8 (wild type)	Mudanjiang 8	67.9	Highly susceptible
2C-50229-5 (*OsWRKY45-1*-knockout)	Dongjin	23.4	Moderately resistant
Dongjin (wild type)	Dongjin	14	Resistant
D115RMH1 (*OsWRKY45-2*-suppressing)	Minghui 63	42.2	Moderately susceptible
D115RMH6 (*OsWRKY45-2*-suppressing)	Minghui 63	44.5	Moderately susceptible
Minghui 63 (wild type)	Minghui 63	32.8	Moderately susceptible

[a]Disease index was calculated with the individual leaf ratings using the following formula: disease index = [sum of numerical ratings from all leaves/(number of leaves assessed × maximum lesion rating)] × 100.

tance was accompanied by increased expression of *OsWRKY13* and markedly induced expression of *PR1a* and *PR1b*. Last, the OsWRKY45-2 protein has four amino acid deletions and six amino acid substitutions compared with OsWRKY45-1, which may result in different three-dimensional structures of the two proteins that preferentially regulate different sets of genes. However, further study is required to ascertain whether the two proteins have different DNA-binding abilities and preferences.

Rice Resistance against *Xoo* and *M. grisea* Is Regulated by Multiple Pathways

Resistance against biotrophic and hemibiotrophic pathogens is usually regulated by the SA-dependent pathway, whereas resistance against necrotrophic pathogens is usually controlled by the JA/ethylene-dependent pathway (Bari and Jones, 2009). SA- and JA/ethylene-dependent defense signals interact with each other either synergistically or antagonistically (Durrant and Dong, 2004). Although *Xoo* and *Xoc* belong to the same species and are both biotrophic pathogens, they have different pathogenic mechanisms. *Xoo* invades rice plants through hydathodes or wounds and lives in the vascular system. *Xoc* penetrates the leaves of rice plants through stomata and wounds and lives in the intercellular spaces of parenchyma and mesophyll cells (Nino-Liu et al., 2006). *M. grisea* is a hemibiotrophic pathogen, which involves initial proliferation inside living host cells before switching to a destructive necrotrophic mode. The infection of *M. grisea* follows a developmental process in the plant surface to leaf epidermal cells (Park et al., 2009).

Rice resistance against the biotrophic pathogen *Xoo* has been reported to be accompanied by increased accumulation of SA and suppressed accumulation of JA (Qiu et al., 2007, 2008; Yuan et al., 2007; Xiao et al., 2009) or by reduced accumulation of both SA and JA (Ding et al., 2008). Our results suggest that OsWRKY45-1 negatively regulated *Xoo* resistance is associated with increased accumulation of SA and JA and OsWRKY45-2 positively regulated *Xoo* resistance is associated with increased accumulation of JA but not SA. These results suggest that multiple mechanisms may be involved in rice resistance against *Xoo*, which may include the antagonistic or synergistic cross talk of SA- and JA-dependent signaling, SA- and JA-independent signaling, and JA-dependent signaling.

A previous study reported that *OsWRKY45* (named *OsWRKY45-1* in this study) acted in an SA signaling pathway that is independent of *NH1*, the rice ortholog of Arabidopsis *NPR1*, in response to the infection of the hemibiotrophic pathogen *M. grisea* (Shimono et al., 2007). Consistently, our results show that *NH1* expression is suppressed in *OsWRKY45-1*-overexpressing plants, which showed enhanced resistance to *M. grisea*. In contrast, *OsWRKY45-1*-overexpressing plants showed significantly suppressed expression of SA- and JA-responsive genes *PR1a*, *PR1b*, and *PR10/PBZ1* and reduced accumulation of SA and JA when without pathogen infection, suggesting that *OsWRKY45-1* positively regulated blast resistance may be independent of SA and JA. The *NH1* expression was also slightly suppressed in *OsWRKY45-2*-overexpressing plants, which showed enhanced resistance to *M. grisea*. However, *OsWRKY45-2*-overexpressing plants showed significantly induced expression of JA synthesis-related genes *PR1a* and *PR1b* and increased accumulation of JA when without pathogen infection, suggesting that *OsWRKY45-2* positively regulated blast resistance may be dependent on JA. This hypothesis is also supported by the report that overexpressing *AOS2* in rice accumulated higher levels of JA, induced expression of *PR1a*, *PR1b*, and *PR10*, and enhanced resistance to *M. grisea* (Mei et al., 2006). Thus, multiple signaling pathways, which are dependent on or independent of SA or JA, may be involved in rice resistance against *M. grisea*.

OsWRKY45 and OsWRKY13 May Regulate Each Other in a Feedback Loop

Activation of OsWRKY13 enhanced rice resistance to both *Xoo* and *M. grisea* (Qiu et al., 2007). The expression patterns of *OsWRKY45* in *OsWRKY13*-overexpressing and -suppressing plants suggest that this pair of alleles function downstream of OsWRKY13 in rice-pathogen interactions (Qiu et al., 2009). In addition, OsWRKY13 bound to *OsWRKY45-1* promoter in vitro (Qiu et al., 2009). Our results here further show that OsWRKY13 also binds to the promoters of *OsWRKY45-1* and *OsWRKY45-2* in vivo, suggesting that OsWRKY13 may directly regulate the expression of this pair of alleles. OsWRKY13 regulates *OsWRKY45-1* and *OsWRKY45-2* perhaps via the binding to W-box, W-box-like, or even other cis-acting elements, because OsWRKY13 also bound to the *OsWRKY45-1* promoter region flanked by PCR primers P45F2 and P45R2, which did not contain W-box or W-box-like element (Fig. 5). Previous studies have reported that a barley (*Hordeum vulgare*) WRKY protein binds to a sugar-responsive cis-element specifically and a tobacco (*Nicotiana tabacum*) WRKY binds to a WK-box (Sun et al., 2003; van Verk et al., 2008). Thus, further study is required to determine whether OsWRKY13 could regulate *OsWRKY45-1* via binding to a non-W-box or non-W-box-like element. Since overexpressing *OsWRKY13* in *japonica* Mudanjiang 8 suppressed *OsWRKY45-1* expression and suppressing *OsWRKY13* in *indica* Minghui 63 increased *OsWRKY45-2* expression (Qiu et al., 2009), OsWRKY13 may function as a transcriptional repressor of *OsWRKY45-1* and *OsWRKY45-2*. The differential expression of *OsWRKY45-1* and *OsWRKY45-2* in rice-*Xoo* interactions may be at least partly due to the binding of OsWRKY13 to different sites of *OsWRKY45-1* and *OsWRKY45-2* promoters. It has been reported that WRKY proteins form homocomplexes or heterocomplexes for DNA binding in pathogen-induced defense responses and other physiological processes (Xie et al., 2006; Xu et al., 2006). OsWRKY13 and other proteins were also detected to bind to the same pathogen-responsive cis-element PRE4 by yeast one-hybrid assay and gel mobility shift assay (Cai et al., 2008). The different preferential binding sites of OsWRKY13 to the promoters of *OsWRKY45-1* and *OsWRKY45-2* may be due to the facts that other activated protein(s) are required for this binding and *indica* and *japonica* backgrounds may have different proteins interacting with OsWRKY13 for DNA binding. Furthermore, the nucleotide difference of the two promoters may result in different three-dimensional DNA structures, which also influence OsWRKY13 binding.

Modulating *OsWRKY45-1* and *OsWRKY45-2* expression also influenced *OsWRKY13* transcript level, suggesting that OsWRKY45-1 and OsWRKY45-2 may also regulate *OsWRKY13* expression at least in rice resistance to *Xoo*. Because *OsWRKY13* expression was suppressed in both *OsWRKY45-1*-overexpressing and -suppressing plants in rice-*Xoo* interactions, other factors in addition to OsWRKY45-1 may contribute to the regulation of *OsWRKY13*. The expression patterns of *OsWRKY13* in *OsWRKY45-2*-overexpressing and -suppressing plants are complementary both without and after *Xoo* infection, suggesting that OsWRKY45-2 may play an important role in the regulation of *OsWRKY13* and that activation of OsWRKY45-2 induces *OsWRKY13* expression. However, further studies are needed to ascertain whether OsWRKY45-1 and OsWRKY45-2 directly or indirectly regulate *OsWRKY13* expression.

CONCLUSION

The *OsWRKY45* alleles, encoding different proteins, play opposite roles in rice resistance against the bacterial pathogens *Xoo* and *Xoc*, but both alleles positively regulate rice resistance against the fungal pathogen *M. grisea*. This pair of alleles regulates rice resistance to the same pathogen via different signaling pathways. These results provide evidence that a pair of allelic defense-responsive genes function oppositely in disease resistance, which may lead us to pay more attention to the roles of this class of genes in host-pathogen interactions caused by allelic diversity. Our results also provide a candidate gene (*OsWRKY45-2*), which regulates a race-nonspecific disease resistance in rice, for breeding programs.

MATERIALS AND METHODS

Gene Isolation, Sequence Comparison, and Allelic Analysis

The genomic fragments of *OsWRKY45-1* and *OsWRKY45-2* genes were amplified from *japonica* rice (*Oryza sativa*) var Nipponbare and *indica* rice var Minghui 63 using primers w45F4 and w45R4 (Supplemental Table S1), respectively, for transformation. The genomic fragments of the genes were sequenced using primers w45F4, w45R4, w45F6, and w45R6 (Supplemental Table S1). Gene structures of *OsWRKY45-1* and *OsWRKY45-2* were determined by alignment of the genomic sequences with the full-length cDNAs AK066255 (http://cdna01.dna.affrc.go.jp/cDNA/) from Nipponbare and EI77K16 (GenBank accession no. CX102514) from Minghui 63 (Zhang et al., 2005). The *OsWRKY45* genes from other *indica* and *japonica* rice lines were also sequenced using primers w45F4, w45R4, w45F6, and w45R6. These lines were *indica* lines Zhenshan 97 and 93-11 and *japonica* lines Mudanjiang 8 and Dongjin. The upstream regions (approximately 1.5 kb upstream of translation start codons) that included the promoters of different *OsWRKY45* alleles were amplified using PCR primers w45F6 and w45R1, and the PCR products were sequenced using primers w45F1, w45F2, w45R1, and P45F2 (Supplemental Table S1).

To determine the allelic relationship of *OsWRKY45-1* and *OsWRKY45-2*, an F2 population, consisting of 146 individuals from a cross between Mudanjiang 8 carrying *OsWRKY45-1* and Minghui 63 carrying *OsWRKY45-2*, was used to map the two genes. A molecular linkage map consisting of 136 markers relatively evenly distributed on 12 rice chromosomes has been constructed using this population (Y. Zhou and S. Wang, unpublished data). A pair of PCR primers, P45F3 and w45R6 (Supplemental Table S1), which generated differently sized PCR products of the two genes, were use as markers. Mapmaker/Exp 3.0 (Lincoln et al., 1992) was used for linkage analysis.

Rice Transformation

The overexpression constructs of *OsWRKY45-1* from *japonica* line Nipponbare and *OsWRKY45-2* from *indica* line Minghui 63 were created by inserting a genomic fragment (2,277 nucleotides for *OsWRKY45-1* and 1,762 nucleotides

for *OsWRKY45-2*) containing the complete gene into vector pU1301, which contained a maize (*Zea mays*) ubiquitin gene promoter (Cao et al., 2007). To construct an RNA interference vector for *OsWRKY45-2*, a 724-nucleotide fragment digested with restriction enzyme *Pst*I from cDNA clone EI77K16 (Zhang et al., 2005) of rice line Minghui 63 was inserted into the pDS1301 vector (Yuan et al., 2007). The recombinant plasmids were introduced into *Agrobacterium tumefaciens* strain EHA105 by electroporation. *Agrobacterium*-mediated transformation was performed using calli derived from mature embryos of Mudanjiang 8 or Minghui 63, according to a published protocol (Lin and Zhang, 2005).

Examination of a Knockout Mutant

Seeds of *OsWRKY45-1* T-DNA insertion mutant 2C-50229 (POSTECH; http://signal.salk.edu/cgi-bin/RiceGE) were kindly provided by professor Gynheung An (Jeong et al., 2006). The background of this mutant is Dongjin (*japonica*). The T-DNA was inserted in the promoter of *OsWRKY45-1*. The insertion site was 401 nucleotides from the translation start codon. The genotype of this mutant was confirmed by PCR amplification using rice primer pair w45F2 and w45R1 (Supplemental Table S1) and T-DNA and rice primer pair RB1 (5′-TTGGGGTTTCTACAGGACGTAAC-3′) and w45R1 (Supplemental Fig. S3, A and B).

Pathogen Inoculation

To examine the resistance of rice plants to bacterial blight disease, plants were inoculated with Philippine *Xanthomonas oryzae* pv *oryzae* strains PXO61 (race 1), PXO86 (race 2), PXO79 (race 3), PXO99 (race 6), and PXO341 (race 10) and with Chinese *Xoo* strains Zhe173 and KS-1-21 at the booting stage by the leaf-clipping method (Chen et al., 2002). Disease was scored by measuring the percentage lesion area (lesion length/leaf length) at 2 to 3 weeks after inoculation. *Xoo* growth rate in rice leaves was determined by counting colony-forming units (Sun et al., 2004).

For blast disease evaluation, seedlings at the three- to four-leaf stage were inoculated with *Magnaporthe grisea* isolate 91-17-2 (kindly provided by Dr. Youliang Peng, China Agricultural University) by the spraying method (Chen et al., 2003). Disease was scored using the 0-to-9 scale rating system (International Rice Research Institute, 2002) at 7 d after inoculation. In this rating system, the disease index, obtained by calculating the number of infected leaves, the number of total screened leaves, and the ratings of infected leaves, was used to evaluate the disease. A disease index greater than or equal to 0 and less than or equal to 5 indicates high resistance, greater than 5 and less than or equal to 15 indicates resistance, greater than 15 and less than or equal to 30 indicates moderate resistance, greater than 30 and less than or equal to 45 indicates moderate susceptibility, greater than 45 and less than or equal to 60 indicates susceptibility, and greater than 60 indicates high susceptibility.

To evaluate the resistance of plants to bacterial streak disease, plants were inoculated with the Chinese *Xanthomonas oryzae* pv *oryzicola* strain RH3 by needle stab method at the booting stage (Chen et al., 2006). Disease was scored by measuring lesion length at 21 d after inoculation. For all the disease evaluations, mock-inoculated (control) plants were treated under the same conditions, except that the pathogen suspension was replaced with water.

Gene Expression Analysis

The qRT-PCR was conducted as described by Qiu et al. (2007). Supplemental Table S1 lists the PCR primers for the genes. To examine the influence of pathogen infection on gene expression, 3-cm leaf fragments next to bacterial infection sites were used for RNA isolation. The expression level of the rice actin gene detected with actin-specific primers was used to standardize the RNA sample for each qRT-PCR. The assays were repeated at least twice, with each repetition having three replicates; similar results were obtained in repeated experiments. SD was calculated for each datum.

Quantification of Hormones

To quantify free SA, the samples were prepared and quantified as described previously (Qiu et al., 2007). In brief, three replicates of each ground sample weighing 300 to 1,000 mg (exact weights were recorded) were used for extraction of SA. The organic extracts containing free SA were quantified using HPLC. SA was detected using a 230-nm wavelength.

To quantify JA, the samples were prepared and quantified as described by Ding et al. (2008). In brief, three replicates of each leaf sample (1 g) were used for JA purification. The purified sample was quantified using the HPLC-electrospray ionization-tandem mass spectrometry system. The quantitative data of JA and 10-dihydro-JA (internal standard; Olchemim) were obtained using the peaks of the precursor ions 209.1 and 211.2 and the product ions 59 and 59, respectively.

Chromatin Immunoprecipitation Assay

Immunoprecipitation was performed as described previously (Benhamed et al., 2006). In brief, 3 g of three- to four-leaf-stage rice seedlings grown on Murashige and Skoog medium were used for sample preparation. Sonicated chromatin fragments were immunoprecipitated with OsWRKY13-specific antibody, which was custom synthesized by NewEast Biosciences against peptide LEVPEPEPEQESEP (201 to 214 of OsWRKY13). The samples were reverse-cross-linked for 6 to 8 h at 65°C and then treated with proteinase K to remove all proteins. DNA was purified by phenol-chloroform extraction and recovered by ethanol precipitation in the presence of 1 μg of glycogen. The precipitated chromatin was resuspended in 50 μL of buffer containing 10 mM Tris-Cl, pH 7.5, and 1 mM EDTA and was used for real-time PCR analysis. An aliquot of nonimmunoprecipitated and sonicated chromatin was reverse-cross-linked for use as the total input DNA control. Immunoprecipitated DNA was analyzed by PCR analysis. PCR was performed in 20 μL with 1 μL of immunoprecipitated DNA and promoter-specific primers (Supplemental Table S1).

Sequence data from this article can be found in the GenBank/EMBL data libraries under accession numbers GQ331930 (Mudanjiang 8), GQ331931 (Dongjin), and GQ331932 (Nipponbare) for *OsWRKY45-1* and GQ331927 (Minghui 63), GQ331928 (Zhenshan 97), and GQ331929 (93-11) for *OsWRKY45-2*.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. The location of *OsWRKY45-1* and *OsWRKY45-2* on the molecular linkage map.

Supplemental Figure S2. Alignment of the promoter regions of *OsWRKY45-1* and *OsWRKY45-2*.

Supplemental Figure S3. Overexpressing *OsWRKY45-1* increased susceptibility to *Xoo* strain PXO61.

Supplemental Figure S4. *OsWRKY45-1* knockout mutant 2C-50229.

Supplemental Figure S5. Overexpressing *OsWRKY45-2* enhanced rice resistance to *Xoo* strain PXO61.

Supplemental Figure S6. The quality of anti-OsWRKY13 antibody was examined using *OsWRKY13*-overexpressing and *OsWRKY13*-suppressing plants.

Supplemental Table S1. Primers used for PCR amplification.

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