Molecular Plant • Volume 1 • Number 3 • Pages 459–470 • May 2008 RESEARCH ARTICLE

Stress- and Pathogen-Induced *Arabidopsis* WRKY48 is a Transcriptional Activator that Represses Plant Basal Defense

Deng-Hui Xing[a,b,2], Zi-Bing Lai[a,2], Zu-Yu Zheng[a], K. M. Vinod[a], Bao-Fang Fan[a] and **Zhi-Xiang Chen[a,1]**

a Department of Botany and Plant Pathology, Purdue University, West Lafayette, Indiana 47907–2054, USA
b Present address: Department of Botany, Miami University, Oxford, Ohio 45056, USA

ABSTRACT Plant WRKY transcription factors can function as either positive or negative regulators of plant basal disease resistance. *Arabidopsis WRKY48* is induced by mechanical and/or osmotic stress due to infiltration and pathogen infection and, therefore, may play a role in plant defense responses. WRKY48 is localized to the nucleus, recognizes the TTGACC W-box sequence with a high affinity in vitro and functions in plant cells as a strong transcriptional activator. To determine the biological functions directly, we have isolated loss-of-function T-DNA insertion mutants and generated gain-of-function transgenic overexpression plants for *WRKY48* in *Arabidopsis*. Growth of a virulent strain of the bacterial pathogen *Pseudomonas syringae* was decreased in the *wrky48* T-DNA insertion mutants. The enhanced resistance of the loss-of-function mutants was associated with increased induction of salicylic acid-regulated *PR1* by the bacterial pathogen. By contrast, transgenic *WRKY48*-overexpressing plants support enhanced growth of *P. syringae* and the enhanced susceptibility was associated with reduced expression of defense-related *PR* genes. These results suggest that WRKY48 is a negative regulator of *PR* gene expression and basal resistance to the bacterial pathogen *P. syringae*.

INTRODUCTION

Plants have evolved sophisticated defense mechanisms that are activated in response to pathogen attack. Upon infection by a virulent pathogen, basal defense mechanisms can be activated by microbe-derived molecules such as bacterial flagellin and lipopolysaccharides, collectively called pathogen or microbe-associated molecular patterns (PAMPs) (Jones and Dangl, 2006). Gram-negative bacterial pathogens such as *Pseudomonas syringae* can deliver effector proteins to plant cells to interfere with PAMP-triggered resistance to promote pathogen virulence. Some of the effectors are specifically recognized by plant resistance proteins and activate strong effector-triggered resistance (Jones and Dangl, 2006). Both PAMP- and effector-triggered resistance are associated with extensive transcriptional reprogramming of plant host genes.

A large body of evidence has shown that WRKY transcription factors play important roles in regulation of genes associated with plant defense responses (Eulgem and Somssich, 2007). First, pathogen infection or treatment with pathogen elicitors or salicylic acid (SA) induces rapid expression of *WRKY* genes from a number of plants (Rushton et al., 1996; Eulgem et al., 1999; Chen and Chen, 2000; Dellagi et al., 2000; Hara et al., 2000; Dong et al., 2003; Kalde et al., 2003; Eckey et al., 2004; Kim and Zhang, 2004; Turck et al., 2004). In *Arabidopsis*, expression of 49 out of 72 tested *WRKY* genes was differentially regulated after pathogen infection or SA treatment (Dong et al., 2003). Secondly, a number of defense or defense-related genes, including several well studied *Pathogenesis-Related* (*PR*) genes and the regulatory *NPR1* gene, contain TTGACC/T W-box elements in their promoters (Rushton et al., 1996; Willmott et al., 1998; Yang et al., 1999; Yu et al., 2001; Turck et al., 2004; Yamamoto et al., 2004; Rocher et al., 2005). A number of studies have shown that these W-box sequences are specifically recognized by WRKY proteins and are necessary for the inducible expression of these genes (Rushton et al., 1996; Willmott et al., 1998; Yang et al., 1999; Yu et al., 2001; Turck et al., 2004; Yamamoto et al., 2004; Rocher et al., 2005). Microarray analysis of gene-expression changes in *Arabidopsis* under different systemic acquired resistance (SAR)-inducing or -repressing conditions with 10 000 ESTs has provided additional evidence that WRKY proteins play important roles in regulating expression of SAR-associated genes (Maleck et al., 2000). A group of 26 genes

[1] To whom correspondence should be addressed. E-mail zhixiang@purdue.edu, fax 765-494-5896.

[2] These two authors contributed equally to this work.

doi: 10.1093/mp/ssn020, Advance Access publication 8 May 2008

including *PR1* was identified to be coordinately induced by various pathogens and defense-inducing conditions. Within the 1.1-kb regions upstream of the predicated translation start sites, only the binding site for WRKY proteins (W-boxes; TTGAC) were found in all 26 promoters, with an average of 4.3 copies per promoter that are often organized in clusters (Maleck et al., 2000). By contrast, a randomly selected set of genes contained, on average, fewer than two W-boxes per promoter (Maleck et al., 2000).

More recent studies have provided direct evidence for the involvement of specific WRKY proteins in plant defense responses. For example, *Arabidopsis WRKY22* and *WRKY29* are induced by a MAPK pathway that confers resistance to both bacterial and fungal pathogens and expression of *WRKY29* in transiently transformed leaves led to reduced disease symptoms (Asai et al., 2002). In tobacco, virus-induced silencing of three *WRKY* genes compromises *N*-gene-mediated resistance to tobacco mosaic virus (Liu et al., 2004). It has been shown that mutations of *Arabidopsis WRKY70* enhances plant susceptibility to both biotrophic and necrotrophic pathogens, including the bacterial pathogen *Erwinia carotovora* as well as fungal pathogens *Erysiphe cichoracearum* and *Botrytis cinerea* (Li et al., 2004; AbuQamar et al., 2006; Li et al., 2006). In addition, *wrky70* mutants are compromised in both basal and resistance gene (*RPP4*)-mediated disease resistance to the oomycete *Hyaloperonospora parasitica* (Knoth et al., 2007). Thus, some of the pathogen-induced WRKY proteins function as important positive regulators of plant disease resistance.

Other WRKY proteins can function as negative regulators of plant disease resistance. For example, mutations of *Arabidopsis WRKY7*, *WRKY11*, and *WRKY17* enhance basal plant resistance to virulent *P. syringae* strains (Park et al., 2005; Journot-Catalino et al., 2006; Kim et al., 2006). Likewise, mutations of *Arabidopsis WRKY25* enhance tolerance to *P. syringae* and overexpression of either *WRKY25* or closely related *WRKY33* enhances susceptibility to the bacterial pathogen and suppresses SA-regulated *PR1* gene expression (Zheng et al., 2006, 2007). The structurally related WRKY18, WRKY40, and WRKY60 also function partially redundantly as negative regulators in plant resistance against the biotrophic bacterial pathogen *P. syringae* and fungal pathogen *E. cichoracearum* (Xu et al., 2006; Shen et al., 2007). In addition, barley HvWRKY1 and HvWRKY2 function as PAMP-inducible suppressors of basal defense (Shen et al., 2007). The WRKY domain in WRKY52/RRS1 R protein that confers resistance toward the bacterial pathogen *Ralstonia solanacearum* may also play a negative role in defense signaling (Noutoshi et al., 2005). The diverse roles of WRKY proteins may reflect the complex signaling and transcriptional networks of plant defense that require tight regulation and fine-tuning.

In *Arabidopsis*, there are more than 70 genes encoding WRKY proteins (Eulgem et al., 2000; Dong et al., 2003). In a previous study, we have analyzed expression of 72 identified *WRKY* genes and found that 49 of them were differentially regulated in response to pathogen infection and SA treatment (Dong et al., 2003). WRKY48 is one those genes that are highly responsive to an avirulent strain of *P. syringae* but not to SA treatment. In the present study, we show that *WRKY48* is a stress- and pathogen-induced gene encoding a nuclear localized WRKY protein with strong DNA-binding and transcriptional activation activities. Growth of a virulent strain of the bacterial pathogen *P. syringae* was reduced in the *wrky48* T-DNA insertion mutants, while transgenic *WRKY48*-overexpressing plants support enhanced growth of *P. syringae.* The altered resistance of the loss-of-function mutants and overexpression lines was associated with altered induction of SA-regulated *PR* genes by the bacterial pathogen. These results strongly suggest that pathogen-induced WRKY48 functions as a negative regulator of defense-related *PR* genes and resistance to the bacterial pathogen *P. syringae*.

RESULTS

Protein Structure, DNA Binding and Subcellular Localization

Arabidopsis WRKY48 (At5g49520) encodes a protein of 399 amino acid residues with a predicted molecular weight of 44.726 kD and a predicted isoelectric point of 6.5143 (Figure 1A). Based on the number and structure of WRKY domains, WRKY48 is classified as a group II WRKY protein. The N-terminus of WRKY48 contains a glutamine-rich domain (Figure 1A) that is found in some transcriptional activators in multi-cellular organisms such as Sp1 (Escher et al., 2000). The glutamine-rich activation domain of Sp1 selectively binds and targets core components of the transcriptional machinery such as TFIID, a multi-protein complex composed of the TATA-box binding protein (TBP) and TBP-associated factors (Escher et al., 2000). WRKY48 also contains a number of serine tracts and, therefore, may be regulated by protein phosphorylation by protein kinases.

A number of isolated WRKY proteins have been shown to bind the TTGACC/T sequence (W-box) (Rushton et al., 1996; Chen and Chen, 2000; Yu et al., 2001). To examine the DNA-binding activity of WRKY48, we expressed the gene in *E. coli*, purified the recombinant protein, and assayed its binding to an oligo DNA molecule with W-box elements (Figure 1B). As shown in Figure 1C, the purified recombinant WRKY48 protein bound the oligo DNA molecule with two direct TTGACC repeats (Pchn0). When directly compared with other recombinant *Arabidopsis* WRKY proteins at the same protein concentrations (WRKY18, 40, 60, 25, 26, 33, and 51), WRKY48 had the highest in-vitro binding activity for the oligo DNA molecule (data not shown). Thus, WRKY48 binds W-box sequences with a high affinity in vitro. To determine whether the TTGACC sequence is essential for the sequence-specific binding activity, we tested a mutant probe (mPchn0) in which the two TTGACC sequences were changed to TTGAAC (Figure 1B). As shown in Figure 1C, binding of WRKY48 to the mutant probe was drastically reduced. Thus, binding of WRKY48 to the TTGACC W-box sequence is highly specific.

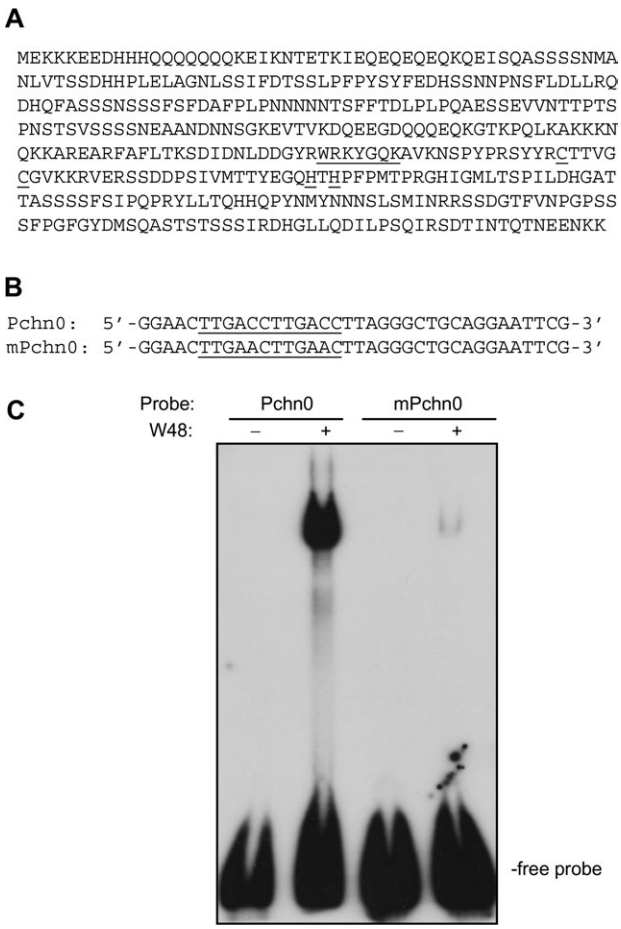

Figure 1. Sequences and DNA-Binding of WRKY48.

(A) Amino acid sequence of WRKY48. The highly conserved WRKYGQK sequences and the residues forming the C_2HC zinc-fingers are underlined.

(B) Sequences of the Pchn0 probe that contains two direct W-box repeats and the mPchn0 probe with the TTGACC sequences mutated into TTGAAC. Underlining signifies W-box sequences.

(C) Sequence-specific binding of Pchn0 by the WRKY48 recombinant protein. Change of the TTGACC to TTGAAC in the mPchn0 probe drastically reduced the binding. No retarded bands were detected in the absence of the recombinant WRKY48 protein.

WRKY48, as a putative transcription factor, is likely to be localized in the nucleus. To test this, we constructed a GFP protein fusion of WRKY48 and demonstrated that this transiently expressed WRKY48-GFP fusion protein was localized exclusively to the nuclei of onion (*Allium cepa*) epidermal cells (Figure 2). By contrast, the GFP protein was found in both the nucleus and cytoplasm because of its small size (Figure 2). Nuclear localization of the WRKY protein supports its role as a transcriptional regulator.

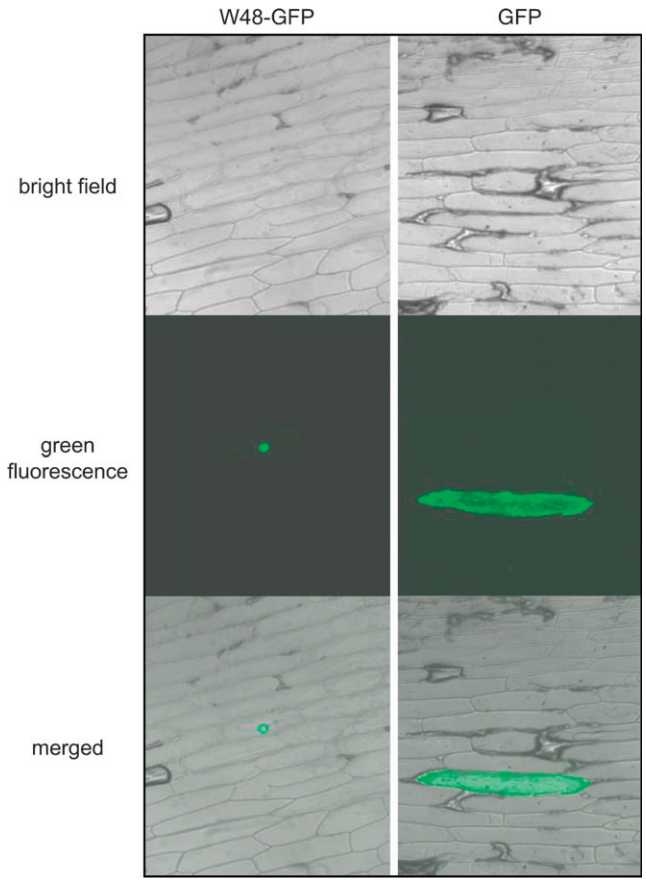

Figure 2. Subcellular Localization of WRKY48.

WRKY48 was fused to GFP to yield W48-GFP. The chimeric protein was localized to the nucleus of onion epidermal cells. GFP alone was detected in both the nucleus and the cytoplasm due to its small size. Bright-field images of the onion epidermal cells are shown in the top panel.

Analysis of *WRKY48* Expression

We have previously shown that *WRKY48* is rapidly induced in *Arabidopsis* plants upon infection by an avirulent strain of *P. syringae* (Dong et al., 2003). To analyze the role of WRKY48 in plant basal defense, we investigated the expression of *WRKY48* after inoculation with the virulent *P. syringae* pv. *tomato* strain DC3000 (*Pst*DC3000). As shown in Figure 3A, *WRKY48* transcript levels increased rapidly in wild-type plants after infiltration with either the control $MgCl_2$ solution (mock inoculation) or the bacterial suspension. However, *WRKY48* induction was stronger in pathogen-infected plants than in $MgCl_2$-treated control plants (Figure 3A). In the four independent experiments performed, *WRKY48* transcripts in *Pst*DC3000-inoculated plants were higher than those in mock-inoculated plants at 2 h post infiltration (hpi) or at both 2 and 4 hpi (Figure 3). After rapid induction during the first 4 h after mock or pathogen inoculation, the levels of *WRKY48* transcripts declined and, in three of the four experiments, reached near basal levels by 24 hpi (Figure 3). *WRKY48*

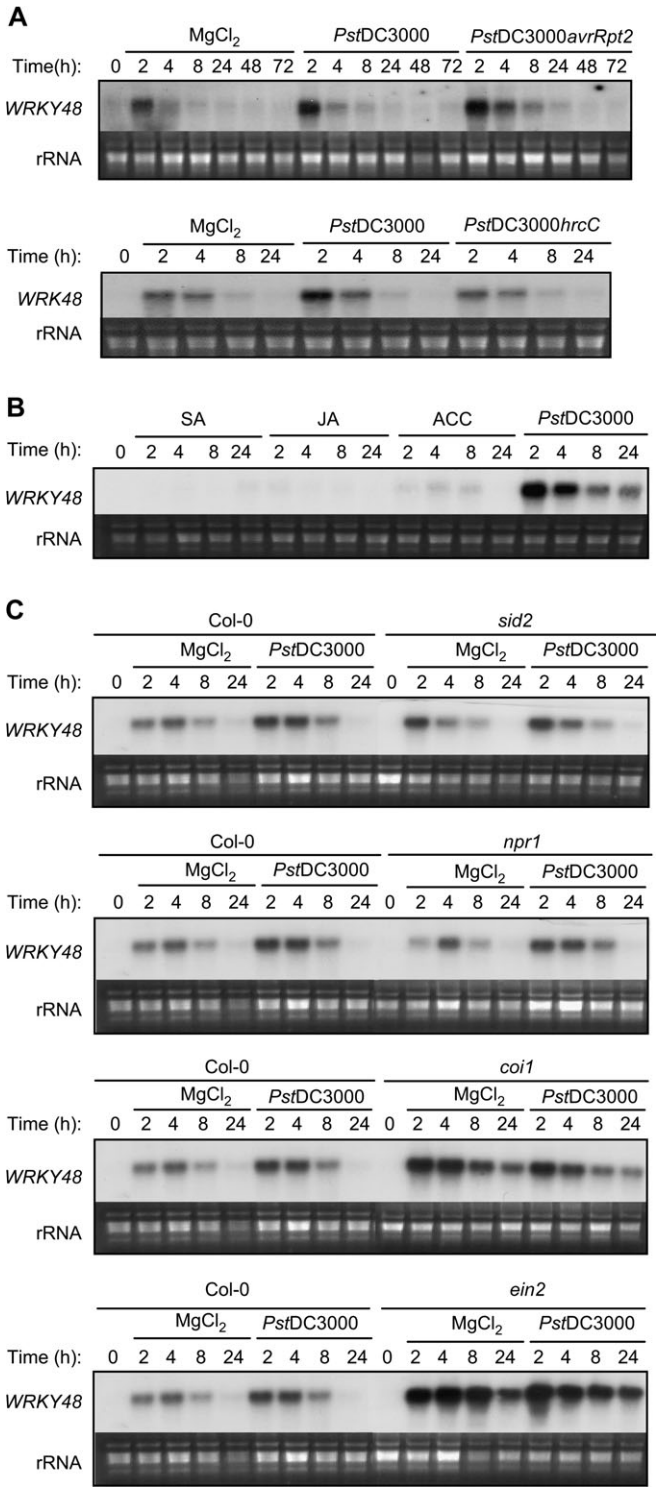

Figure 3. Expression of *WRKY48*.

(A) Time course of expression of WRKY48 after mock and pathogen inoculation. Five-week-old *Arabidopsis* plants (Col-0) were infiltrated with 10 mM $MgCl_2$, *Pst*DC3000, *Pst*DC3000*avrRpt2* or *Pst*DC3000*hrcC* (OD_{600} = 0.001 in 10 mM $MgCl_2$). The infiltrated leaves were collected at indicated times after inoculation for RNA isolation. RNA gel blot analysis was performed with a ^{32}P-labeled *WRKY48* probe. Ethidium bromide staining of rRNA is shown for the assessment of equal loading.

(B) Expression of *WRKY48* after chemical treatments or pathogen infection. Five-week-old wild-type (Col-0) plants were sprayed with SA (1 mM), ACC (0.1 mM), or MethylJA (0.1 mM) or infiltrated with *Pst*DC3000 (OD_{600} = 0.001 in 10 mM $MgCl_2$). Leaf collection, RNA isolation and RNA blot analysis of *WRKY48* expression were performed as in (A).

(C) Expression of *WRKY48* after mock or pathogen infection in defense signaling mutants. Five-week-old *Arabidopsis* wild-type (Col-0) and mutant plants were infiltrated with 10 mM $MgCl_2$ or *Pst*DC3000 (OD_{600} = 0.001 in 10 mM $MgCl_2$). The infiltrated leaves were collected at indicated times after inoculation for RNA isolation. RNA gel blot analysis was performed with a ^{32}P-labeled *WRKY48* probe.

These experiments were repeated three times, with similar results.

induction after infection by the avirulent *Pst*DC3000avrRpt2 strain was similar to or slightly stronger than that after infection by the virulent *Pst*DC3000 strain (Figure 3A). On the other hand, the *Pst*DC3000*hrcC* mutant, which is defective in the type III secretion system (Deng et al., 1998; Deng and Huang, 1999), did not enhance *WRKY48* transcript levels above those in $MgCl_2$-treated control plants (Figure 3A). Thus, the bacterial type III secretion system plays an important role in pathogen-enhanced expression of *WRKY48*. We also analyzed the induction of *WRKY48* by defense-inducing molecules SA, 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor of ethylene (ET) biosynthesis, and methyl jasmonic acid (JA). Unlike *Pst*DC3000, these defense-inducing molecules induced little expression of *WRKY48* (Figure 3B).

To determine whether stress- and pathogen-induced *WRKY48* expression is mediated by the SA, ET, and/or JA signaling pathways, stress- and pathogen-induced *WRKY48* expression was monitored in various signaling mutants. $MgCl_2$- and pathogen-induced *WRKY48* expression was not significantly affected in the *sid2* and *npr1-3* mutants, which are defective in SA biosynthesis and signaling, respectively (Cao et al., 1997; Wildermuth et al., 2001) (Figure 3C). The levels of *WRKY48* transcripts were higher in the JA-insensitive *coi1-1* mutant plants (Xie et al., 1998) than those in the wild-type plants (Figure 3C). In addition, while *WRKY48* transcripts were detected at 24 hpi in wild-type plants only in one of the four experiments, they were present at substantial levels in the *coi1* mutant plants at this hpi in all four experiments (Figure 3C). The enhanced and prolonged induction of *WRKY48* by $MgCl_2$ and *Pst*DC3000 was even more pronounced in the ET-insensitive *ein2-1* mutant (Alonso et al., 1999) (Figure 3C). These results suggest that JA and ET signaling pathways have a negative role in stress- and pathogen-induced *WRKY48* expression.

WRKY48 is a Transcriptional Activator

To determine the transcriptional regulatory activity of WRKY48 in planta, we employed a previously established transgenic system in which the transcriptional regulatory activity of a protein is determined through assays of a reporter gene in stably transformed plants (Kim et al., 2006). The reporter gene in the system is a *GUS* gene driven by a synthetic promoter consisting of the –100 minimal *CaMV 35S* promoter and eight copies of the *LexA* operator sequence (Figure 4A). Transgenic *Arabidopsis* plants harboring the reporter gene constitutively expressed similarly low levels of the *GUS* reporter gene due to the minimal *35S* promoter used, thereby making them useful for assays of transcription activation or repression by determining increase or decrease in GUS activities following co-expression of an effector protein.

To generate the WRKY48 effector, we fused its coding sequence with that of the DNA-binding domain (DBD) of LexA (Figure 4A). The fusion construct was subcloned behind the steroid-inducible *Gal4* promoter in pTA7002 (Aoyama and Chua, 1997) and transformed into transgenic plants that already contain the *GUS* reporter construct. Unfused *WRKY48* and *LexA DBD* genes were also subcloned into pTA7002 and transformed into transgenic *GUS* reporter plants as controls (Figure 4A). Transgenic plants containing both the reporter and an effector construct were identified through antibiotic resistance screens. To determine how the effectors influence *GUS* reporter gene expression, we determined the changes of GUS activities in these transgenic plants following induction of the effector gene expression by spraying 20 μM dexamethasone (DEX), a steroid. In the transgenic plants that expressed unfused WRKY48 or LexA DBD effector, the ratios of GUS activities measured before DEX treatment to those measured after DEX treatment were close to 1 (Figure 4B). These results indicated that induced expression of *WRKY48* or *LexA DBD* alone had no significant effect on expression of the *GUS* reporter gene. In the transgenic plants harboring the *LexA DBD-WRKY48* effector gene, induction of the fusion effector after DEX treatment resulted in an ~25-fold increase in GUS activity (Figure 4B). These results strongly suggest that WRKY48 is a transcriptional activator in plant cells.

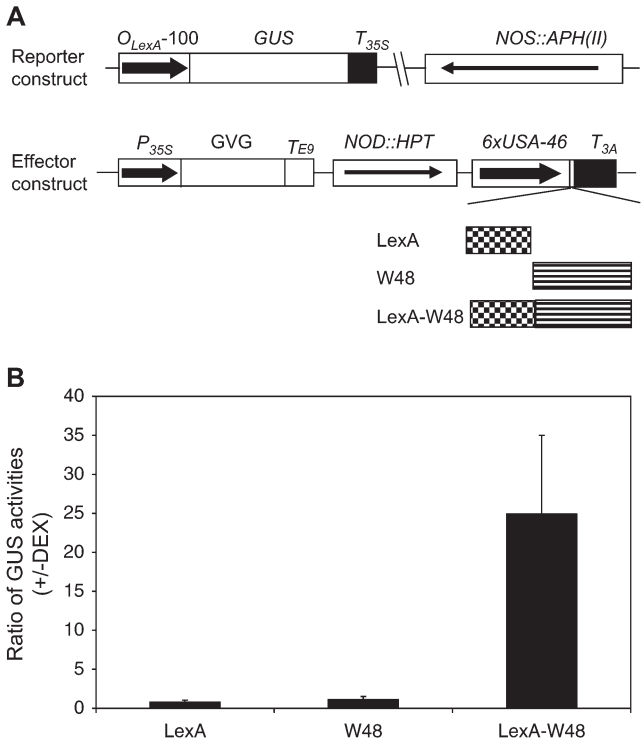

Figure 4. Transcriptional Activation Activity of WRKY48.

(A) Constructs of reporter and effector genes. The *GUS* reporter gene is driven by a synthetic promoter consisting of the –100 minimal CaMV *35S* promoter and eight copies of the *LexA* operator sequence. The effector genes were cloned into pTA7002 behind the steroid-inducible promoter. The three effector genes encode LexA DBD (LexA), WRKY48 (W48), and LexADBA-WRKY48 fusion protein (LexA-W48), respectively.

(B) Effects on *GUS* reporter gene expression by induced expression of effector genes. The ratios of GUS activities were calculated from the GUS activities determined in the leaves harvested 18 h after DEX treatment (+) over those determined prior to DEX treatment (–). Only those transformants that displayed induced expression of the effector genes as determined from RNA blotting following DEX treatment were used in the analyses. The means and errors were calculated from at least 15 positive transformants. The experiments were repeated once, with similar results.

Disrupting or Altering *WRKY48* Expression Affect Basal Disease Resistance

To analyze its biological roles directly, we identified and characterized two T-DNA knockout mutants for *WRKY48*. Both *wrky48-1* (Salk_066438) and *wrky48-2* (Sail_1267_D04) contain a T-DNA insertion in the first exon of the *WRKY* gene (Figure 5A). Homozygous mutant plants were identified by PCR with *WRKY48*-specific primers flanking the insertion sites. Northern blotting analysis confirmed the absence of the normal, full-length *WRKY48* transcript in pathogen-infected mutant plants (Figure 5B). The *wrky48* mutants grew and flowered at the same rates as the wild-type plants and exhibited no detectable alteration in morphology.

To determine possible altered phenotypes in disease resistance, we examined the response of wild-type and *wrky48* mutant plants to *Pst*DC3000, which is virulent on *Arabidopsis* ecotype Columbia (Whalen et al., 1991). Plants were inoculated with the bacteria and the growth of the pathogen was monitored. At 1 and 2 dpi, there was a marked decrease (approximately nine-fold) in the bacterial growth in the *wrky48* mutants when compared with the wild-type plants (Figure 6A). The reduced bacterial growth during the first 2 d after inoculation was recovered substantially during

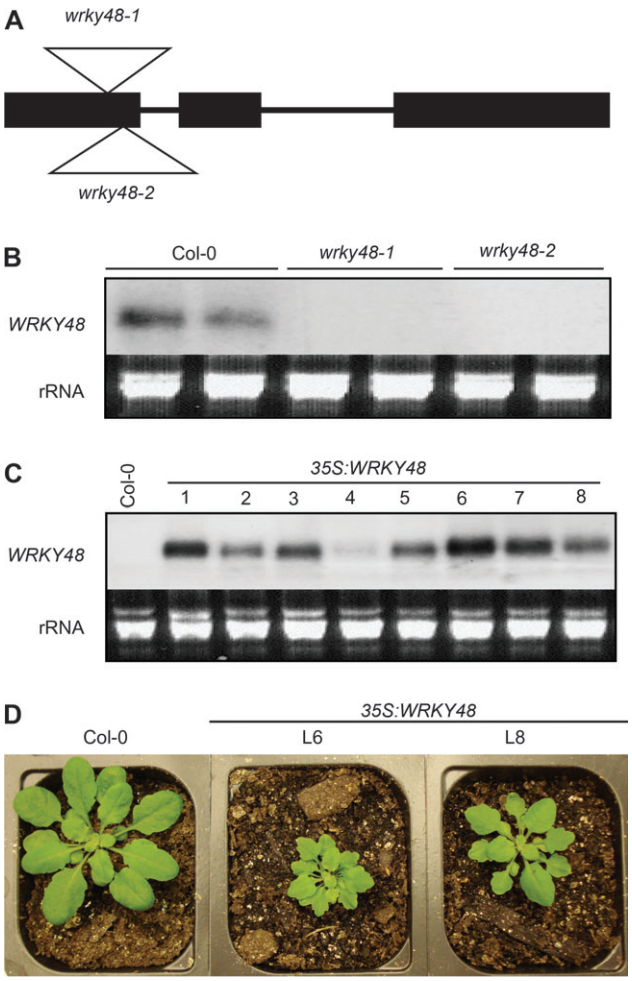

Figure 5. T-DNA Insertion Mutants and Overexpression Lines for *WRKY48*.

(A) Diagram of *WRKY48* and the *wrky48-1* and *wrky48-2* T-DNA insertion mutants.

(B) RNA gel blot analysis of *wrky48-1* and *wrky48-2* mutants. Wild-type and mutant plants were infiltrated with *Pst*DC3000 (OD_{600} = 0.001 in 10 mM $MgCl_2$). The leaves were harvested at 2 hpi and total RNA was isolated. After separation on the gels and blotting to nylon membranes, the blots were probed with a *WRKY48*-specific DNA fragment.

(C) *WRKY48* expression in transgenic plants. RNA samples were prepared from leaves of 5-week-old wild-type (Col-0) and transgenic plants and probed with a *WRKY48*-specific probe. Transgenic *WRKY48* lines 6 and 8 contained a single T-DNA insertion in the genome and exhibited stable *WRKY48* expression. The F3 homozygous progeny plants were used in all the experiments in the study.

(D) Morphology of representative 4-week-old wild-type (Col-0) and transgenic plant lines 6 (L6) and 8 (L8) over-expressing *WRKY48*.

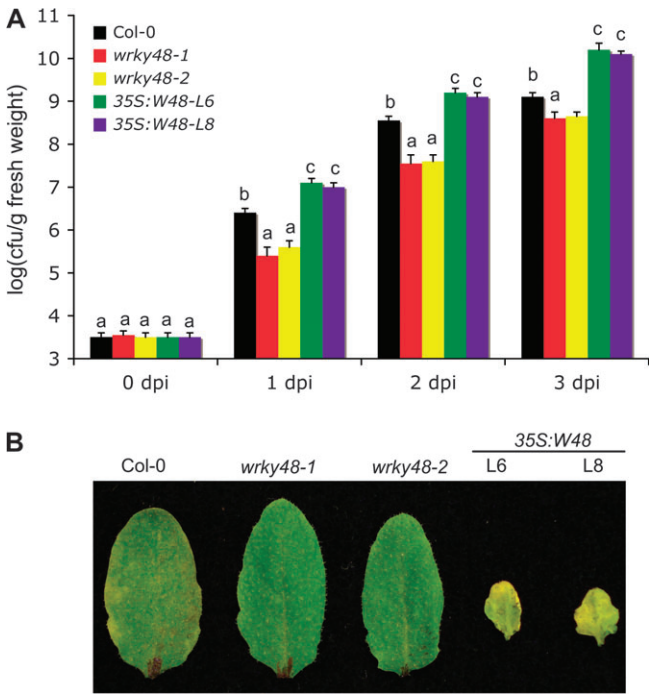

Figure 6. Altered Responses of T-DNA Insertion Mutants and Overexpression Plants to *Pst*DC3000.

(A) Altered bacterial growth. Wild-type (Col-0), mutants and overexpression plants for *WRKY48* were infiltrated with a suspension of *Pst*DC3000 (OD_{600} = 0.001 in 10 mM $MgCl_2$). Samples were taken at 0, 1, 2, and 3 dpi to determine the growth of the bacterial pathogen. The means and standard errors were calculated from 10 plants for each treatment. According to Duncan's multiple range test (P = 0.05), means of colony-forming units (cfu) do not differ significantly at the same dpi if they are indicated with the same letter.

(B) Altered disease symptom development. Pathogen inoculation of wild-type (Col-0), mutant and overexpression plants was performed as in (A). Pictures of representative inoculated leaves taken at 3 dpi.

These experiments were repeated three times, with similar results.

the third day, as there was only a three- to five-fold difference in bacterial titers between wild-type and *wrky48* mutants at 3 dpi (Figure 6A). Nonetheless, the inoculated leaves of the *wrky48* mutant consistently displayed less chlorosis than wild-type plants at 3 dpi (Figure 6C). Thus, disruption of *WRKY48* resulted in enhanced resistance, particularly during the early stages after infection, to the bacterial pathogen.

We also attempted to express *WRKY48* constitutively in transgenic *Arabidopsis* plants. A full-length cDNA for *WRKY48* was placed behind the *CaMV 35S* promoter (*35S:WRKY48*) and transformed into *Arabidopsis*. Northern blotting showed that several transgenic plants contained elevated levels of the *WRKY48* transcript even in the absence of pathogen infection

(Figure 5C). Those plants that constitutively express *WRKY48* exhibited significantly smaller plant sizes and more serrated leaves than the wild-type (Figure 5D). A similar alteration in growth and morphology has been previously observed in transgenic *Arabidopsis* plants that constitutively express pathogen-induced *WRKY18* (Chen and Chen, 2002). Two transgenic lines with constitutive *WRKY48* expression and a single T-DNA locus in their genomes were identified from the ratio of antibiotic resistance phenotypes and chosen for further analysis.

There was a marked increase (~8–15-fold) in bacterial growth in the transgenic *35S:WRKY48* overexpression lines when compared with the wild-type plants (Figure 6A). The transgenic plants also developed more severe disease symptoms than the wild-type plants after infection by the bacterial pathogen (Figure 6B). Thus, constitutive overexpression of *WRKY48* led to increased growth of the bacterial pathogen and enhanced development of disease symptoms in the transgenic plants.

Expression of Defense-Related Genes

To further analyze the defense responses in the loss-of-function mutants and overexpression lines for *WRKY48*, we compared their defense gene expression with that of wild-type plants after pathogen infection. First, we examined pathogen-induced expression of *PR1*. The wild-type, transgenic *35S:WRKY48* plants and the *wrky48-1* mutant plants were inoculated with *Pst*DC3000. Total RNA was isolated from the inoculated leaves harvested at 0 and 1 dpi and probed with a *PR1* gene probe. At 0 dpi, no *PR1* transcripts were detected in wild-type, *wrky48* mutants or *WRKY48*-overexpressing plants (Figure 7A). At 1 dpi, *PR1* transcripts were detected in both wild-type and *wrky48* mutant plants but the level was significantly enhanced in the mutants when compared to those in the wild-type plants (Figure 7A). By contrast, the level of the *PR1* transcripts was decreased in the transgenic *35S:WRKY48* plants when compared with that in the wild-type plants (Figure 7A).

We also compared the wild-type, transgenic *35S:WRKY48* plants and the *wrky48* mutants for SAR-associated defense gene expression. Three lower leaves of plants were first inoculated with an avirulent strain of *P. syringae* (*Pst*DC3000*avrRpt2*) and total RNA was isolated from upper leaves at 1, 2, and 3 dpi. As shown in Figure 7B, in SAR-induced wild-type plants, transcripts for *PR* genes started to increase at 1 dpi, peaked at 2 dpi and declined at 3 dpi. In the *wrky48* mutants, a similar trend was observed but the transcript levels of the *PR1*, *PR2*, and *PR5* genes were significantly higher than those in the wild-type plants (Figure 7B). By contrast, induction of these *PR* genes was greatly reduced in the upper leaves of the transgenic *35S:WRKY48* plants after inoculation of their lower leaves with the avirulent bacterial strain. These results indicated that overexpression of *WRKY48* blocked *PR* gene expression while knockout of the *WRKY* gene enhanced induction of these *PR* genes during the development of SAR.

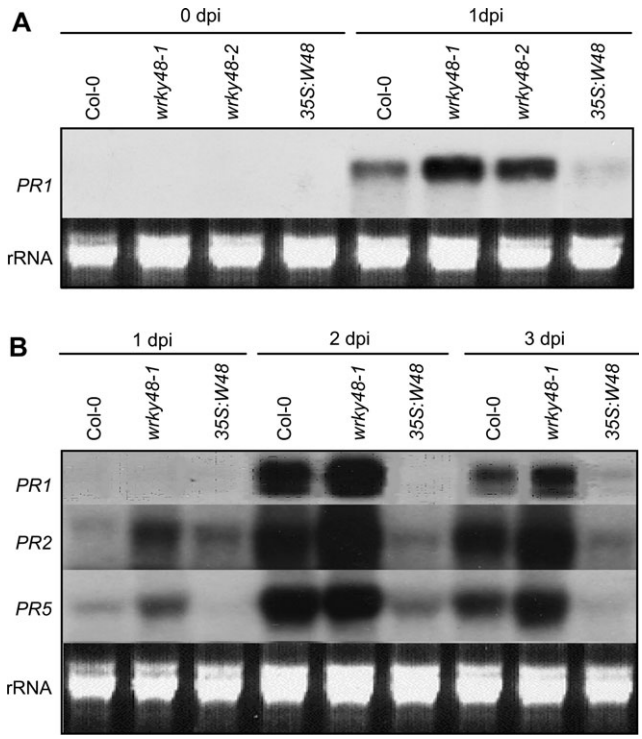

Figure 7. Pathogen-Induced Defense Gene Expression.

(A) Wild-type (Col-0), *wrky48-1*, *wrky48-2*, and transgenic *35S:WRKY48* plants were inoculated with *Pst*DC3000 (OD_{600} = 0.001). Total RNA was isolated from inoculated leaves harvested at 1 dpi, separated on an agarose (1.2%)-formaldehyde gel and probed with a *PR-1* fragment. The ethidium bromide stain of rRNA is shown for each lane to allow assessment of equal loading.

(B) Three lower leaves of wild-type (Col-0), *wrky48-1*, and transgenic *35S:WRKY48* plants were inoculated with *Pst*DC3000 expressing *AvrRpt2* (OD_{600} = 0.01). Total RNA was isolated from three upper uninoculated leaves harvested at indicated times, separated on an agarose (1.2%)-formaldehyde gel. Three separate blots were prepared from the same preparation of total RNA and probed with the *PR1, PR2*, and *PR5* probes, respectively. The ethidium bromide stain of rRNA from one of the three blots is shown for each lane to allow assessment of equal loading.

DISCUSSION

WRKY48 as a Negative Regulator of Plant Basal Defense

Arabidopsis WRKY48 is a stress- and pathogen-induced *WRKY* gene that encodes a nuclear localized WRKY transcriptional activator with a high binding affinity for DNA molecules with W-box sequences (Figures 1–3). Through analysis of both transgenic overexpressing lines and T-DNA insertion mutants, we have provided strong evidence that this pathogen-induced *WRKY* gene functions as a negative regulator of plant basal disease resistance. In the *wrky48* knockout mutants, the

growth of the bacterial pathogen was reduced, particularly during the first 2 d after inoculation, when compared with that in the wild-type plants (Figure 6A). The stronger effect on the bacterial growth during the early stage of infection is likely a reflection of the rapid but transient expression of *WRKY48* in pathogen-infected plants (Figure 1A). We have also shown that constitutive overexpression of *WRKY48* led to enhanced susceptibility to the bacterial pathogen *P. syringae* as manifested by enhanced growth of the bacterial pathogen and development of disease symptoms (Figure 6). It should be noted that overexpression of *WRKY48* resulted in significantly smaller size and more serrated leaves of transgenic plants (Figure 5D). In a previously reported study, we have shown that overexpression of another pathogen-induced *WRKY* gene, *WRKY18*, also resulted in significantly smaller plant size and more serrated leaves of transgenic plants (Chen and Chen, 2002). However, unlike transgenic *35S:WRKY48* plants, transgenic *35S:WRKY18* plants constitutively express *PR* genes at their mature stages and were more resistant to the bacterial pathogen *P. syringae* (Chen and Chen, 2002). Therefore, the more susceptible phenotype of the transgenic *35S:WRKY48* plants is unlikely to be caused by their altered growth and morphology.

It is tempting to speculate why plants activate expression of a negative regulatory gene in response to pathogen infection that would make them more susceptible to the invading pathogen. This could simply reflect the fact that the defense mechanisms induced in plants upon pathogen infection are a massive network of different pathways comprising both positive and negative regulators. Negative regulators may play roles in preventing over-activation of defense mechanisms that could be detrimental to other biological processes important for plant growth and development, thereby reducing the cost of plant defense to overall plant fitness. In addition, different defense mechanisms mediated through different signaling pathways may have differential effects on plant resistance to different types of microbial pathogens (Glazebrook, 2005). Different signaling pathways can exhibit not only positive but also negative interactions, resulting in both cooperative and antagonistic relationships. A negative regulator for one pathway may be important for maintaining proper balancing of a signaling network so that activation of one signaling pathway effective for combating one type of microbial pathogens does not greatly compromise other signaling pathways important for resistance to other types of microbial pathogens.

There is also a possibility that pathogen-induced expression of a negative regulator of plant defense, such as *WRKY48*, may result from the counter-defense mechanism of an invading pathogen. A number of studies have recently shown that gram-negative bacterial pathogens such as *P. syringae* have various mechanisms of suppressing innate immunity of plant hosts (Alfano and Collmer, 2004). For example, a number of type III effector proteins from *P. syringae* suppress hypersensitive cell death of plant host cells (Alfano and Collmer, 2004; Abramovitch and Martin, 2005). A *P. syringae* type III effector can compromise defense-related callose deposition in the host cell wall (Hauck et al., 2003; DebRoy et al., 2004). In addition, *P. syringae* type III effectors and phytotoxin coronatine can augment a COI1-dependent pathway in plants to promote parasitism (He et al., 2004). A number of studies have reported that PAMP-induced MAPK signaling pathways can be inactivated by specific effector proteins of *P. syringae* (He et al., 2006; Zhang et al., 2007). We have found that unlike wild-type virulent and avirulent *Pst*DC3000 strains, the *Pst*DC3000*hrcC* mutant strain defective in the type III secretion system did not enhance *WRKY48* expression (Figure 1A). Thus, certain virulence factors from the bacterial pathogen may actively promote expression of negative regulatory genes such as *WRKY48* as an active counter-defense mechanism to compromise the defense mechanism and promote parasitism.

WRKY48 is rapidly induced by infiltration with $MgCl_2$ (Figure 3), likely due to osmotic and/or mechanical stresses generated from the infiltration. The stress-induced expression suggests that WRKY48 may play a role in plant responses to abiotic stresses. Notably, highest levels of *WRKY48* transcripts were detected during the first few hours after infiltration but then declined steadily, reaching near basal levels by 24 hpi (Figure 3). *WRKY48* induction was substantially enhanced and sustained in the JA and ET signaling mutants (Figure 3), suggesting that ET- and JA-mediated signaling plays a role in suppressing *WRKY48* expression following its rapid induction after pathogen inoculation. By suppressing expression of negative regulators of basal defense such as *WRKY48*, JA and ET may play a positive role in some aspects of *Arabidopsis* responses to *P. syringae*. Several studies have indeed shown that systemic immunity of *Arabidopsis* responses to *P. syringae* is dependent on ET and JA (Verhagen et al., 2004; Ahn et al., 2007; Truman et al., 2007). Both positive and negative regulation of *WRKY48* expression in pathogen-inoculated plants may, therefore, reflect the complex and dynamic nature of plant defense and pathogen counter-defense during plant–pathogen interactions. Upon pathogen inoculation, PAMP-induced defense responses are rapidly activated. At the same time, the osmotic and/or mechanical stresses generated from inoculation induce certain stress-induced regulatory genes such as WRKY48 with a negative role in basal disease resistance. As a counter-defense mechanism, invading pathogens may actively promote expression of such negative regulators through the action of certain secreted effector proteins. To counter the counter-defense mechanism, plants may rely on the ET- and JA-dependent pathways to repress the expression of the negative regulatory genes to promote defense responses.

Mechanisms for the Repression of Plant Basal Defense by WRKY48

SA plays an important role in *Arabidopsis* basal resistance to *P. syringae*, as mutants defective in SA biosynthesis or signaling are more susceptible to the bacterial pathogen than the wild-type plants (Glazebrook, 2005). However, SA accumulation in the *wrky48* mutants is normal (data not shown), suggesting that

the stress- and pathogen-induced WRKY transcription factor does not play an important role in SA biosynthesis. On the other hand, SA-regulated *PR* gene expression was altered in both the transgenic overexpression lines and the T-DNA insertion mutants for *WRKY48* (Figure 7). Expression of the defense-related *PR1* gene was reduced significantly in the leaves of transgenic overexpression plants after infection by a virulent strain of *P. syringae* (Figure 7). Furthermore, unlike in the wild-type plants, where infection of lower leaves by an avirulent strain of *P. syringae* led to induction of *PR* genes in upper uninoculated leaves, this SAR-associated *PR* gene expression was almost completely abolished in the transgenic *35S:WRKY48* plants (Figure 7B). By contrast, expression of *PR* genes during pathogen-induced SAR was enhanced in the *wrky48* mutant plants when compared with that in wild-type plants (Figure 7B). These results strongly suggest that WRKY48 is a negative regulator of pathogen-induced *PR* gene expression.

Several previous studies have indeed suggested that WRKY proteins may play negative roles in the regulation of plant defense genes. For example, the *Arabidopsis PR1* gene, a reliable marker for SA-mediated defense responses, contains a W-box sequence in its promoter (Lebel et al., 1998). Mutation of the W-box resulted in enhanced promoter activity when assayed with a reporter gene, suggesting that the W-box sequence acts as a negative *cis*-acting element in the expression of the defense-related gene (Lebel et al., 1998). Likewise, we have shown that a cluster of three W-box sequences in the promoter of the *Arabidopsis WRKY18* gene reduced its promoter activity (Chen and Chen, 2002). Using chromatin immuno-precipitation, it has been shown in cultured parsley cells that the promoter sites of elicitor-induced genes such as *PcWRKY1* are constitutively occupied by certain WRKY proteins but displaced by other WRKY proteins in a stimulus-dependent manner (Turck et al., 2004). It appears that different WRKY proteins act in a mutually competing manner with dynamic displacement upon pathogen infection or elicitor treatment. We have recently reported that WRKY7 from *Arabidopsis*, like WRKY48, is a negative regulator of plant defense gene expression and basal resistance to *P. syringae* based on the phenotypes of both loss-of-function mutants and overexpression lines (Kim et al., 2006). *Arabidopsis* WRKY7 is a transcriptional repressor in plant cells and, therefore, may function as a negative regulator of plant basal defense by directly repressing expression of plant defense genes (Kim et al., 2006).

Although it suppresses disease resistance and defense gene expression (Figures 6 and 7), WRKY48 acts as a transcriptional activator in plant cells (Figure 3). Thus, WRKY48 does not appear to repress defense genes directly. Instead, WRKY48 may first activate certain unknown negative regulators that, in turn, repress defense genes. Further characterization of in-vivo binding sites and identification of downstream target genes of WRKY48 will provide valuable insights into how the pathogen-induced transcription factor negatively regulates plant *PR* gene expression and compromise disease resistance to the bacterial pathogen.

METHODS

Materials

[α-^{32}P]dATP (>3000 Ci mmol^{-1}) was obtained from New England Nuclear; other common chemicals were purchased from Sigma. *Arabidopsis* plants were grown in a growth chamber at 22°C and 150 μEm^{-2}s^{-1} light on a photoperiod of 12 h light and 12 h dark. SA was dissolved in water as 100 mM stock solutions and adjusted to pH 6.5 with KOH. SA, MeJA, and ACC treatments were performed by spraying the plants with solutions at indicated concentrations.

Recombinant Protein and DNA-Binding

Preparation of recombinant WRKY48 proteins and DNA-binding assays were performed as previously described (Yu et al., 2001).

Subcellular Localization

Onion epidermal cell layers were peeled and placed inside up on the MS plates. Plasmid DNAs of appropriate fusion genes (0.5 μg) were introduced to the onion cells using a pneumatic particle gun (PDS 1000, Du Pont). The condition of bombardment was a vacuum of 28 inch Hg, helium pressure of 1100 or 1300 psi, and 6 cm of target distance using 1.1 μm of tungsten microcarriers. After bombardment, tissues were incubated on the MS plates for 24 h at 22°C. Samples were observed directly or transferred to glass slides.

Pathogen Infection

Pathogen inoculations were performed by infiltration of leaves of at least six plants for each treatment with the *P. syringae* pv. *tomato* DC3000 strain (OD_{600} = 0.001 in 10 mM $MgCl_2$). Inoculated leaves were harvested at indicated dpi and homogenized in 10 mM $MgCl_2$. Diluted leaf extracts were plated on King's B medium supplemented with rifampicin (100 μg ml^{-1}) and kanamycin (25 μg ml^{-1}) and incubated at 25°C for 2 d before counting the colony-forming units.

Northern Blot Analysis

For Northern blot analysis of plant gene expression, total RNA was isolated from treated leaves using the TRIZOL reagent (BRL Life Technologies, Rockville, MD). The RNA was separated on agarose (1.2%)-formaldehyde gels and blotted onto nylon membranes. Hybridization was performed with random-primed ^{32}P-labeled DNA probes in PerfectHyb plus hybridization buffer (Sigma) overnight at 68°C. The membranes were washed at 68°C once in 2 SSC and 0.1% SDS for 5 min, twice in 0.5 SSC and 0.1% SDS for 20 min and once in 0.1 SSC and 0.1% SDS for 20 min. DNA probes for *WRKY48* were isolated from its full-length cDNA clone. DNA probes for *PR* genes were prepared from PCR-amplified DNA fragments using the following gene-specific primers: *PR1*: 5′-TTCTTCCCTCGAAAGCTCAA-3′/5′-CGTTCACATAATTCCCACGA-3′; *PR2*: 5′-TGGTGTCAGATTCCGGTACA-3′/5′-TCGGTGATCCATTCTTCACA-3′; *PR5*: 5′-GCGATGGAGGATTTGAATTG-3′/5′-GCGTAGCTATAGGCGTCAGG-3′.

Assays of Transcriptional Regulatory Activity of WRKY48

Transgenic *Arabidopsis* plants containing a *GUS* reporter gene driven by a synthetic promoter consisting of the –100 minimal *CaMV 35S* promoter and eight copies of the *LexA* operator sequence were previously described (Kim et al., 2006). To generate effector genes, the DNA fragment for the LexA DBD was digested from the plasmid pEG202 (Clontech) using *Hind*III and *Eco*RI and cloned into the same sites in pBluescript. The full-length *WRKY48* cDNA fragment was subsequently subcloned behind the *LexA DBD* to generate translational fusion. The *LexA DBD-WRKY48* fusion gene was cloned into the *Xho*I and *Spe*I site of pTA2002 behind the steroid-inducible promoter (Aoyama and Chua, 1997). As controls, the unfused *LexADBD* and *WRKY48* genes were also cloned into the same sites of PTA7002. These effector constructs were directly transformed into the transgenic *GUS* reporter plants and double transformants were identified through screening for antibiotic (hygromycin) resistance. Determination of activation or repression of *GUS* reporter gene expression by the effector proteins was performed as previously described (Kim et al., 2006).

Identification of the *wrky48* T-DNA Insertion Mutants

The *wrky48-1* (Salk_066438) and *wrky48-2* (Sail_1267_D04) each contain a T-DNA insertion in the first exon the *WRKY48* gene. Confirmation of the T-DNA insertions was done by performing PCR using a combination of a gene-specific primer and a T-DNA border primer. The nature and location of the T-DNA insertions were confirmed by sequencing the PCR products. Homozygous *wrky48* mutant plants were identified by PCR using a pair of primers corresponding to sequences flanking the T-DNA insertion sites (pW48F: 5′-CCCTTTTGCTCTTGTTGTTGA-3′ and pW48R: 5′-TCAGATCATCATCCGTTGGA-3′). To remove additional T-DNA loci or mutations from the mutants, backcrosses to wild-type plants were performed and plants homozygous for the T-DNA insertion were again identified.

Construction of Transgenic Overexpression Lines

To generate the *35S:WRKY48* construct, the cDNA fragment that contained the full coding sequence and 3′ untranslated region of *WRKY48* was excised with *KpnI* and *SalI* from a cloning plasmid and subcloned into the same restriction sites of the *Agrobacterium* transformation vector pOCA30 (Chen and Chen, 2002) in the sense orientation behind the *CaMV 35S* promoter. *Arabidopsis* transformation was performed by the floral dip procedure (Clough and Bent, 1998). The seeds were collected from the infiltrated plants and selected in MS medium containing 50 μg ml^{-1} kanamycin. Kanamycin-resistant plants were transferred to soil 9 d later and grown in a growth chamber.

Accession Numbers

The Arabidopsis Genome Initiative identifiers for the genes described in this article are as follows: WRKY48 (At5g49520), PR1(At2g14610), PR2 (At3g57260) and PR5 (At1g75040).

FUNDING

This work was supported in part by the US National Science Foundation grant MCB-0209819. This is journal paper 2007–18186 of the Purdue University Agricultural Research Program.

ACKNOWLEDGMENTS

We would like to thank RIKEN Bioresource Center for the full-length cDNA clone for *WRKY48* and the *Arabidopsis* Resource Center at the Ohio State University and Syngenta Biotechnology, Inc. for the *wrky48* T-DNA insertion mutants. We would also like to thank Drs Shengyang He (Michigan State University, East Lansing, Michigan) and Xiaoyan Tang (Kansas State University, Manhattan, MA) for the *Pst*DC3000*hrcC* mutant and Dr Walter Gassmann (University of Missouri, Columbia, MO) for other *P. syringae* strains.
No conflict of interest declared.

REFERENCES

Abramovitch, R.B., and Martin, G.B. (2005). AvrPtoB: a bacterial type III effector that both elicits and suppresses programmed cell death associated with plant immunity. FEMS Microbiol. Lett. **245,** 1–8.

AbuQamar, S., Chen, X., Dhawan, R., Bluhm, B., Salmeron, J., Lam, S., Dietrich, R.A., and Mengiste, T. (2006). Expression profiling and mutant analysis reveals complex regulatory networks involved in Arabidopsis response to Botrytis infection. Plant J. **48,** 28–44.

Ahn, I.P., Lee, S.W., and Suh, S.C. (2007). Rhizobacteria-induced priming in Arabidopsis is dependent on ethylene, jasmonic acid, and NPR1. Mol. Plant Microbe Interact. **20,** 759–768.

Alfano, J.R., and Collmer, A. (2004). Type III secretion system effector proteins: double agents in bacterial disease and plant defense. Annu. Rev. Phytopathol. **42,** 385–414.

Alonso, J.M., Hirayama, T., Roman, G., Nourizadeh, S., and Ecker, J.R. (1999). EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis. Science. **284,** 2148–2152.

Aoyama, T., and Chua, N.-H. (1997). A glucocorticoid-mediated transcriptional induction system in transgenic plants. Plant J. **11,** 605–612.

Asai, T., Tena, G., Plotnikova, J., Willmann, M.R., Chiu, W.L., Gomez-Gomez, L., Boller, T., Ausubel, F.M., and Sheen, J. (2002). MAP kinase signalling cascade in Arabidopsis innate immunity. Nature. **415,** 977–983.

Cao, H., Glazebrook, J., Clarke, J.D., Volko, S., and Dong, X. (1997). The Arabidopsis NPR1 gene that controls systemic acquired resistance encodes a novel protein containing ankyrin repeats. Cell. **88,** 57–63.

Chen, C., and Chen, Z. (2000). Isolation and characterization of two pathogen- and salicylic acid- induced genes encoding WRKY DNA-binding proteins from tobacco. Plant Mol. Biol. **42,** 387–396.

Chen, C., and Chen, Z. (2002). Potentiation of developmentally regulated plant defense response by AtWRKY18, a pathogen-induced Arabidopsis transcription factor. Plant Physiol. **129,** 706–716.

Clough, S.J., and Bent, A.F. (1998). Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. **16,** 735–743.

DebRoy, S., Thilmony, R., Kwack, Y.B., Nomura, K., and He, S.Y. (2004). A family of conserved bacterial effectors inhibits salicylic acid-mediated basal immunity and promotes disease necrosis in plants. Proc. Natl Acad. Sci. U S A. **101,** 9927–9932.

Dellagi, A., Helibronn, J., Avrova, A.O., Montesano, M., Palva, E.T., Stewart, H.E., Toth, I.K., Cooke, D.E., Lyon, G.D., and Birch, P.R. (2000). A potato gene encoding a WRKY-like transcription factor is induced in interactions with Erwinia carotovora subsp. atroseptica and Phytophthora infestans and is coregulated with class I endochitinase expression [In Process Citation]. Mol. Plant Microbe Interact. **13,** 1092–1101.

Deng, W.L., and Huang, H.C. (1999). Cellular locations of Pseudomonas syringae pv. syringae HrcC and HrcJ proteins, required for harpin secretion via the type III pathway. J. Bacteriol. **181,** 2298–2301.

Deng, W.L., Preston, G., Collmer, A., Chang, C.J., and Huang, H.C. (1998). Characterization of the hrpC and hrpRS operons of Pseudomonas syringae pathovars syringae, tomato, and glycinea and analysis of the ability of hrpF, hrpG, hrcC, hrpT, and hrpV mutants to elicit the hypersensitive response and disease in plants. J. Bacteriol. **180,** 4523–4531.

Dong, J., Chen, C., and Chen, Z. (2003). Expression profile of the Arabidopsis WRKY gene superfamily during plant defense response. Plant Mol. Biol. **51,** 21–37.

Eckey, C., Korell, M., Leib, K., Biedenkopf, D., Jansen, C., Langen, G., and Kogel, K.H. (2004). Identification of powdery mildew-induced barley genes by cDNA-AFLP: functional assessment of an early expressed MAP kinase. Plant Mol. Biol. **55,** 1–15.

Escher, D., Bodmer-Glavas, M., Barberis, A., and Schaffner, W. (2000). Conservation of glutamine-rich transactivation function between yeast and humans. Mol. Cell Biol. **20,** 2774–2782.

Eulgem, T., and Somssich, I.E. (2007). Networks of WRKY transcription factors in defense signaling. Curr. Opin. Plant Biol. **10,** 366–371.

Eulgem, T., Rushton, P.J., Robatzek, S., and Somssich, I.E. (2000). The WRKY superfamily of plant transcription factors. Trends Plant Sci. **5,** 199–206.

Eulgem, T., Rushton, P.J., Schmelzer, E., Hahlbrock, K., and Somssich, I.E. (1999). Early nuclear events in plant defence signalling: rapid gene activation by WRKY transcription factors [In Process Citation]. Embo J. **18,** 4689–4699.

Glazebrook, J. (2005). Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. Annu. Rev. Phytopathol. **43,** 205–227.

Hara, K., Yagi, M., Kusano, T., and Sano, H. (2000). Rapid systemic accumulation of transcripts encoding a tobacco WRKY transcription factor upon wounding. Mol. Gen. Genet. **263,** 30–37.

Hauck, P., Thilmony, R., and He, S.Y. (2003). A Pseudomonas syringae type III effector suppresses cell wall-based extracellular defense in susceptible Arabidopsis plants. Proc. Natl Acad. Sci. U S A. **100,** 8577–8582.

He, P., Chintamanani, S., Chen, Z., Zhu, L., Kunkel, B.N., Alfano, J.R., Tang, X., and Zhou, J.M. (2004). Activation of a COI1-dependent pathway in Arabidopsis by Pseudomonas syringae type III effectors and coronatine. Plant J. **37,** 589–602.

He, P., Shan, L., Lin, N.C., Martin, G.B., Kemmerling, B., Nurnberger, T., and Sheen, J. (2006). Specific bacterial suppressors of MAMP signaling upstream of MAPKKK in Arabidopsis innate immunity. Cell. **125,** 563–575.

Jones, J.D., and Dangl, J.L. (2006). The plant immune system. Nature. **444,** 323–329.

Journot-Catalino, N., Somssich, I.E., Roby, D., and Kroj, T. (2006). The transcription factors WRKY11 and WRKY17 act as negative regulators of basal resistance in Arabidopsis thaliana. Plant Cell. **18,** 3289–3302.

Kalde, M., Barth, M., Somssich, I.E., and Lippok, B. (2003). Members of the Arabidopsis WRKY group III transcription factors are part of different plant defense signaling pathways. Mol. Plant Microbe Interact. **16,** 295–305.

Kim, C.Y., and Zhang, S. (2004). Activation of a mitogen-activated protein kinase cascade induces WRKY family of transcription factors and defense genes in tobacco. Plant J. **38,** 142–151.

Kim, K.C., Fan, B., and Chen, Z. (2006). Pathogen-induced Arabidopsis WRKY7 is a transcriptional repressor and enhances plant susceptibility to Pseudomonas syringae. Plant Physiol. **142,** 1180–1192.

Knoth, C., Ringler, J., Dangl, J.L., and Eulgem, T. (2007). Arabidopsis WRKY70 is required for full RPP4-mediated disease resistance and basal defense against Hyaloperonospora parasitica. Mol. Plant Microbe Interact. **20,** 120–128.

Lebel, E., Heifetz, P., Thorne, L., Uknes, S., Ryals, J., and Ward, E. (1998). Functional analysis of regulatory sequences controlling PR-1 gene expression in Arabidopsis. Plant J. **16,** 223–233.

Li, J., Brader, G., and Palva, E.T. (2004). The WRKY70 transcription factor: a node of convergence for jasmonate-mediated and salicylate-mediated signals in plant defense. Plant Cell. **16,** 319–331.

Li, J., Brader, G., Kariola, T., and Palva, E.T. (2006). WRKY70 modulates the selection of signaling pathways in plant defense. Plant J. **46,** 477–491.

Liu, Y., Schiff, M., and Dinesh-Kumar, S.P. (2004). Involvement of MEK1 MAPKK, NTF6 MAPK, WRKY/MYB transcription factors, COI1 and CTR1 in N-mediated resistance to tobacco mosaic virus. Plant J. **38,** 800–809.

Maleck, K., Levine, A., Eulgem, T., Morgan, A., Schmid, J., Lawton, K.A., Dangl, J.L., and Dietrich, R.A. (2000). The transcriptome of Arabidopsis thaliana during systemic acquired resistance. Nat. Genet. **26,** 403–410.

Noutoshi, Y., Ito, T., Seki, M., Nakashita, H., Yoshida, S., Marco, Y., Shirasu, K., and Shinozaki, K. (2005). A single amino acid insertion in the WRKY domain of the Arabidopsis TIR-NBS-LRR-WRKY-type disease resistance protein SLH1 (sensitive to low humidity 1) causes activation of defense responses and hypersensitive cell death. Plant J. **43,** 873–888.

Park, C.Y., et al. (2005). WRKY group IId transcription factors interact with calmodulin. FEBS Lett. **579,** 1545–1550.

Rocher, A., Dumas, C., and Cock, J.M. (2005). A W-box is required for full expression of the SA-responsive gene SFR2. Gene. **344,** 181–192.

Rushton, P.J., Torres, J.T., Parniske, M., Wernert, P., Hahlbrock, K., and Somssich, I.E. (1996). Interaction of elicitor-induced DNA-binding proteins with elicitor response elements in the promoters of parsley PR1 genes. Embo J. **15,** 5690–5700.

Shen, Q.H., Saijo, Y., Mauch, S., Biskup, C., Bieri, S., Keller, B., Seki, H., Ulker, B., Somssich, I.E., and Schulze-Lefert, P. (2007). Nuclear activity of MLA immune receptors links isolate-specific and basal disease-resistance responses. Science. **315,** 1098–1103.

Truman, W., Bennett, M.H., Kubigsteltig, I., Turnbull, C., and Grant, M. (2007). Arabidopsis systemic immunity uses conserved defense signaling pathways and is mediated by jasmonates. Proc. Natl Acad. Sci. U S A. **104,** 1075–1080.

Turck, F., Zhou, A., and Somssich, I.E. (2004). Stimulus-dependent, promoter-specific binding of transcription factor WRKY1 to Its native promoter and the defense-related gene PcPR1-1 in Parsley. Plant Cell. **16,** 2573–2585.

Verhagen, B.W., Glazebrook, J., Zhu, T., Chang, H.S., van Loon, L.C., and Pieterse, C.M. (2004). The transcriptome of rhizobacteria-induced systemic resistance in arabidopsis. Mol. Plant Microbe Interact. **17,** 895–908.

Whalen, M.C., Innes, R.W., Bent, A.F., and Staskawicz, B.J. (1991). Identification of Pseudomonas syringae pathogens of Arabidopsis and a bacterial locus determining avirulence on both Arabidopsis and soybean. Plant Cell. **3,** 49–59.

Wildermuth, M.C., Dewdney, J., Wu, G., and Ausubel, F.M. (2001). Isochorismate synthase is required to synthesize salicylic acid for plant defence. Nature. **414,** 562–565.

Willmott, R.L., Rushton, P.J., Hooley, R., and Lazarus, C.M. (1998). DNase1 footprints suggest the involvement of at least three types of transcription factors in the regulation of alpha-Amy2/A by gibberellin. Plant Mol. Biol. **38,** 817–825.

Xie, D.X., Feys, B.F., James, S., Nieto-Rostro, M., and Turner, J.G. (1998). COI1: an Arabidopsis gene required for jasmonate-regulated defense and fertility. Science. **280,** 1091–1094.

Xu, X., Chen, C., Fan, B., and Chen, Z. (2006). Physical and functional interactions between pathogen-induced Arabidopsis WRKY18, WRKY40, and WRKY60 transcription factors. Plant Cell. **18,** 1310–1326.

Yamamoto, S., Nakano, T., Suzuki, K., and Shinshi, H. (2004). Elicitor-induced activation of transcription via W box-related cis-acting elements from a basic chitinase gene by WRKY transcription factors in tobacco. Biochim. Biophys. Acta. **1679,** 279–287.

Yang, P., Wang, Z., Fan, B., Chen, C., and Chen, Z. (1999). A pathogen- and salicylic acid-induced WRKY DNA-binding activity recognizes the elicitor response element of the tobacco class I chitinase gene promoter. Plant J. **18,** 141–149.

Yu, D., Chen, C., and Chen, Z. (2001). Evidence for an important role of WRKY DNA binding proteins in the regulation of NPR1 gene expression. Plant Cell. **13,** 1527–1540.

Zhang, J., et al. (2007). A Pseudomonas syringae effector inactivates MAPKs to suppress PAM-induced immunity in plants. Cell Host & Microbe. **1,** 175–185.

Zheng, Z., Mosher, S.L., Fan, B., Klessig, D.F., and Chen, Z. (2007). Functional analysis of Arabidopsis WRKY25 transcription factors in plant defense against Pseudomonas syringae. BMC Plant Biol. **7,** 2.

Zheng, Z., Qamar, S.A., Chen, Z., and Mengiste, T. (2006). Arabidopsis WRKY33 transcription factor is required for resistance to necrotrophic fungal pathogens. Plant J. **48,** 592–605.