The Transcription Factors WRKY11 and WRKY17 Act as Negative Regulators of Basal Resistance in *Arabidopsis thaliana* [W][OA]

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Transcription factors are believed to play a pivotal role in the activation and fine-tuning of plant defense responses, but little is known about the exact function of individual transcription factors in this process. We analyzed the role of the IId subfamily of WRKY transcription factors in the regulation of basal resistance to *Pseudomonas syringae* pv *tomato* (*Pst*). The expression of four members of the subfamily was induced upon challenge with virulent and avirulent strains of *Pst*. Mutant analyses revealed that loss of WRKY11 function increased resistance toward avirulent and virulent *Pst* strains and that resistance was further enhanced in *wrky11 wrky17* double mutant plants. Thus, WRKY11 and WRKY17 act as negative regulators of basal resistance to *Pst*. Genome-wide expression analysis and expression studies of selected genes in single and double mutants demonstrated that both transcription factors modulate transcriptional changes in response to pathogen challenge. Depending on the target gene, WRKY11 and WRKY17 act either specifically or in a partially redundant manner. We demonstrate complex cross-regulation within the IId WRKY subfamily and provide evidence that both WRKY transcription factors are involved in the regulation of *Pst*-induced jasmonic acid–dependent responses. These results provide genetic evidence for the importance of WRKY11 and WRKY17 in plant defense.

INTRODUCTION

The defense responses that plants mount against invading microorganisms are orchestrated by a complex reprogramming of host cells, both at the infection site and in systemic tissues, and rely on major changes in gene expression (Somssich and Hahlbrock, 1998; Maleck et al., 2000; Nimchuk et al., 2003; Tao et al., 2003; De Vos et al., 2005). These responses are highly specific to be effective against each individual species within the panoply of potential plant pathogens, ranging from necrotrophic fungi to biotrophic oomycetes, or from bacteria colonizing the vascular system to root-feeding nematodes. But defense is also intimately connected to general plant physiological and developmental processes to benefit the plant and to minimize the costs associated with resistance (Heil, 2002; Brown, 2003). Thus, induction of the plant defense response does not occur through a linear pathway but by a complex signaling network interconnected by crosstalk to the networks regulating other plant functions (Thomma et al., 2001; Glazebrook et al., 2003; Katagiri, 2004). Two well-studied key mediators of plant resistance are the plant hormones salicylic acid (SA) and jasmonic acid (JA). Research on the plant *Arabidopsis thaliana* has led to a model in which SA primarily activates defense responses to biotrophic pathogens, such as *Hyaloperonospora parasitica* and *Pseudomonas syringae*, whereas JA mainly activates defense responses to necrotrophic pathogens such as *Fusarium oxysporum* (Thomma et al., 2001). Both signaling pathways have been shown to act antagonistically. Mutants affected in SA synthesis or SA signal transduction show enhanced susceptibility to *Pseudomonas syringae* pv *tomato* (*Pst*), whereas JA signal transduction mutants possess enhanced resistance to *Pst* (Delaney et al., 1995; Nawrath and Métraux, 1999; Kloek et al., 2001). In addition, JA inhibits SA-dependent responses and vice versa (Thomma et al., 1998).

The major transcriptional reprogramming associated with the plant defense response requires not only plant hormones but also the action of diverse transcription factors (Chen et al., 2002; Singh et al., 2002; Chen and Zhu, 2004; Eulgem, 2005). However, despite increasing circumstantial evidence implicating different families of transcriptional regulators in this process, little is known about the exact function of individual transcription factors. The WRKY class of transcriptional regulators appears to play a major role in the regulation of plant defense responses. Members of this family are characterized by the presence of one or two highly conserved WRKY domains. This 60–amino acid domain comprises the name-giving, absolutely conserved sequence motif WRKYGQK and a zinc-finger motif. Both conserved elements of the domain are necessary for the high binding affinity of WRKY proteins to the consensus sequence (C/T)TGAC(C/T), named the W-box (Eulgem et al., 2000; Maeo et al., 2001; Zhang and Wang,

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2005). NMR analysis has shown that WRKY domains form a four-stranded β-sheet that is stabilized by a zinc binding pocket localized at one end of the β-sheet, and it is likely that the conserved WRKYGQK sequence directly binds the W-box consensus motif (Yamasaki et al., 2005).

There are 74 WRKY proteins in *Arabidopsis* that have been classified into three groups according to the number and the type of their WRKY domains (Eulgem et al., 2000). Group III members contain a single C-C-H-C zinc finger, group II proteins contain a single C-C-H-H zinc finger, and group I proteins contain two C-C-H-H zinc fingers. Group II is further divided into subgroups according to the level of homology within the WRKY domain and the presence of additional domains. For example, the proteins belonging to the IId subfamily possess three domains in common in addition to the WRKY domain: a putative nuclear localization signal, the so-called HARF domain of unknown function, and the C domain, which has been shown to mediate a calcium-dependent interaction with calmodulin (Park et al., 2005).

W-boxes are a major class of *cis*-acting elements that confer pathogen and elicitor inducibility, either on their own, when coupled to minimal promoters, or in the context of promoters of pathogen- or elicitor-responsive genes such as pathogenesis-related proteins, receptor protein kinases, or WRKY transcription factors (Rushton et al., 1996; Wang et al., 1998; Eulgem et al., 1999; Yang et al., 1999; Du and Chen, 2000; Robatzek and Somssich, 2002; Rushton et al., 2002). As shown by different transcriptome analyses, W-boxes are overrepresented in the promoters of *Arabidopsis* genes that are upregulated during resistance (*R*) gene–mediated resistance, basal defense, elicitor responses, and systemic acquired resistance (Maleck et al., 2000; Eulgem et al., 2004; Navarro et al., 2004; Zipfel et al., 2004). These data suggest that WRKY proteins are central regulators of the pathogen-induced active defense response. This notion is also supported by the finding that the majority of *Arabidopsis* WRKY genes are upregulated in defense responses or after treatment with defense-inducing elicitors or hormones (Chen et al., 2002; Kalde et al., 2003). In one expression profiling study, 49 of 72 tested *Arabidopsis* WRKY genes were induced in response to treatment with SA or *Pst* (Dong et al., 2003). The importance of WRKY factors in regulating the plant defense transcriptome is further underlined by studies using overexpression and gene-silencing approaches. Overexpression of the pathogen-induced *WRKY18* gene in *Arabidopsis* leads to an amplification of developmentally regulated defense responses (Chen and Chen, 2002). Overexpression and antisense depletion of *WRKY70* has provided compelling evidence for a central role of this factor in integrating signals from the antagonistic JA and SA signaling pathways (Li et al., 2004).

Apparent functional redundancy has severely hampered attempts to genetically define the roles of individual WRKY transcription factors in the regulation of plant defense (Ulker and Somssich, 2004; Eulgem, 2005). Recently, however, Li et al. (2006) demonstrated that loss of WRKY70 function impaired plant resistance toward the fungal pathogen *Erysiphe cichoracearum*, and Xu et al. (2006) established a function for the homologous genes *WRKY18*, *WRKY40*, and *WRKY60* in resistance to *Pst* and *Botrytis cinerea* by direct genetic means.

In this study, the seven members of the *Arabidopsis* WRKY IId subfamily were analyzed for their role in resistance to *Pst*. We provide genetic evidence that two of them, WRKY11 and WRKY17, act as negative regulators of basal resistance to the bacterial pathogen. These two regulators show partial redundancy in the transcriptional reprogramming that occurs in response to pathogen challenge. In addition, WRKY11 and WRKY17 are shown to be involved in the regulation of *Pst*-induced responses that are JA-dependent. Finally, our results reveal complex cross-regulation within the IId WRKY subfamily.

RESULTS

Four Members of the WRKY IId Subfamily Are Induced in Response to *Pseudomonas*

Transcriptome analysis of *hxc2* (Godard et al., 2000), an *Arabidopsis* mutant affected in specific resistance to *Xanthomonas campestris* pv *campestris* and basal resistance to *Pst*, revealed the downregulation of one member of the IId subfamily of WRKY proteins, *WRKY11*, in the mutant compared with wild-type plants. To evaluate the role of the IId subfamily of WRKY proteins (Figure 1A) in the regulation of *Arabidopsis* defense responses, the expression of all seven members of this subfamily was analyzed after challenge with virulent or avirulent strains of *Pst* DC3000 by quantitative real-time PCR (Q-RT-PCR). In untreated leaves or leaves treated with water, *WRKY7*, *WRKY11*, *WRKY15*, *WRKY17*, *WRKY21*, and *WRKY39* are weakly expressed, whereas *WRKY74* is barely detectable (data not shown). After inoculation with virulent *Pst* DC3000 or avirulent *Pst* DC3000 expressing the avirulence gene *avrRpt2*, the expression of *WRKY7*, *WRKY11*, *WRKY15*, and *WRKY17* is strongly induced, whereas the expression of *WRKY21*, *WRKY39*, and *WRKY74* is not altered significantly (Figure 1B; data not shown for WRKY74, which is below the detection limit). *WRKY11*, *WRKY7*, and *WRKY17* show similar expression profiles, with a rapid and transient induction that peaks 2 h after inoculation. Induction in response to virulent bacteria is similar to the induction in response to avirulent bacteria, but slightly weaker. The expression profile of *WRKY15* in response to *Pst* is different. In the incompatible interaction with *Pst* DC3000 (*avrRpt2*), induction is rapid and sustained, whereas in the compatible interaction with *Pst* DC3000, it is delayed but increases steadily between 6 and 12 h after inoculation.

Many pathogen-responsive genes are also induced by a wide range of abiotic stresses. This has been shown for some WRKY transcription factors, for which induction by wounding, drought, and cold has been documented (Hara et al., 2000; Chen et al., 2002; Lee et al., 2005; Taki et al., 2005). Therefore, it was important to evaluate the specificity of the induction of the WRKY IId gene members in the pathogen response. For this purpose, we analyzed the responsiveness of *WRKY11*, *WRKY17*, *WRKY7*, and *WRKY15* to various abiotic stresses, including wounding, cold, high salt concentration, and drought, as well as to treatment with the stress hormone abscisic acid. None of these treatments led to a strong induction of any of the WRKY IId genes (data not shown for *WRKY7* and *WRKY15* or for drought and abscisic acid treatments for *WRKY11* and *WRKY17*). We observed a weak
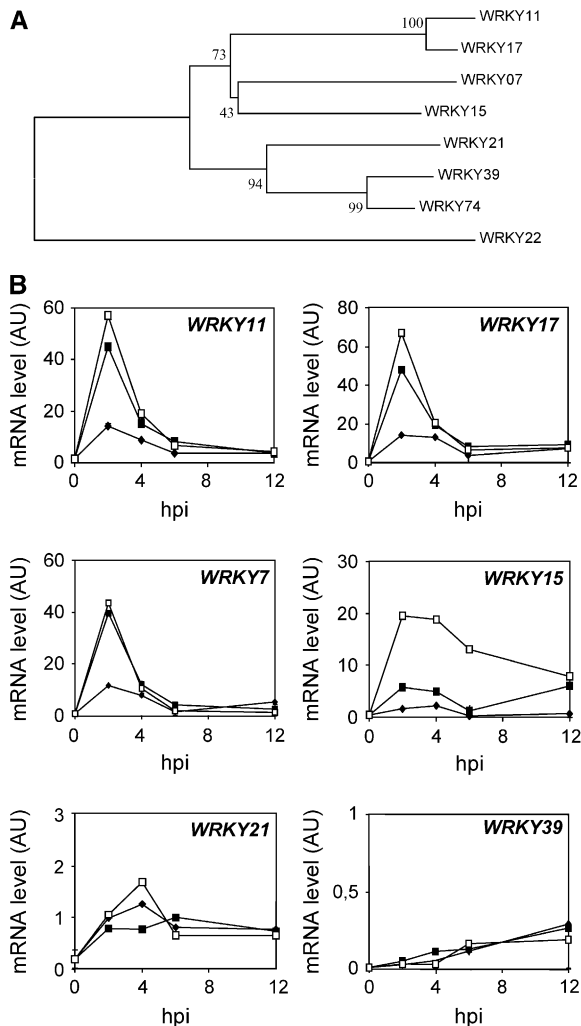
A

B

Figure 1. The Pathogen-Responsive Expression Pattern of WRKY IId Subfamily Members.

(A) The phylogenetic tree of the WRKY IId subfamily was generated using the neighbor-joining method after alignment of the entire amino acid sequences of the IId subfamily members WRKY7, WRKY15, WRKY11, WRKY17, WRKY21, WRKY39, and WRKY74 and the IIe subfamily member WRKY22 with ClustalW (see Supplemental Figure1 online). Distance in the tree corresponds to evolutionary distance. Bootstrap values are indicated at the nodes of the tree. Tree building with the maximum-parsimony, minimum-evolution, and UPGMA methods gave similar results.

(B) Transcript levels of the *WRKY* genes belonging to the IId subfamily were determined by Q-RT-PCR with cDNA generated from leaves of 4-week-old Col-0 plants inoculated with water (closed diamonds), *Pst* DC3000 (closed squares), and *Pst* DC3000 (*avrRpt2*) (open squares) at 10^7 colony-forming units (cfu)/mL. The expression values of individual genes were normalized using the expression level of β-*Tubulin4* as an internal standard. Mean expression values were calculated from the results of two independent experiments. *WRKY74* mRNA levels are not shown because they are below the level of detection. hpi, hours after inoculation; AU, arbitrary units.

induction (fourfold) of *WRKY11* after cold treatment and salt treatment and a rapid and transient induction of *WRKY11* between 30 min and 1 h after wounding (threefold) (see Supplemental Figure 1 online). However, these activations are considerably weaker than those observed in response to inoculation with *Pst* (>30-fold), indicating that *WRKY11*, *WRKY17*, *WRKY7*, and *WRKY15* are specifically upregulated in response to pathogen challenge and are not general stress response genes.

wrky11 but Not *wrky7* or *wrky17* Mutants Show Enhanced Basal Resistance

To study the function of the *WRKY* IId genes *WRKY11*, *WRKY17*, *WRKY7*, and *WRKY15* in the regulation of defense responses, we searched *Arabidopsis* mutant collections for loss-of-function insertion mutants. For *WRKY11*, we identified two alleles that we named *wrky11.1* and *wrky11.2* (Figure 2). *wrky11.1* was isolated from the ZIGIA En-insertion population (Wisman et al., 1998). This mutant carries a 4-bp insertion in the 5′ part of the coding sequence, resulting in a premature stop codon at position 121 in the predicted amino acid sequence of *WRKY11* and thus a truncated gene product. By Q-RT-PCR, we detected the transcript at a slightly but significantly lower level than in wild-type plants (see Supplemental Figure 2 online). The second allele, *wrky11.2*, is from the GABI collection (Li et al., 2003; Rosso et al., 2003) and carries an insertion within the third and last exon, just after the position encoding the first Cys of the zinc finger motif (Figure 2). By Q-RT-PCR analysis, we verified disruption of the gene. Primer pairs positioned 3′ to the insertion site or around the insertion site did not amplify any product, whereas primer pairs in the 5′ part of the transcript amplified a product showing that a truncated mRNA is produced. As disruption of the zinc finger motif has been shown to completely abolish the W-box–specific DNA binding activity of WRKY transcription factors (Maeo et al., 2001), it is very likely that *wrky11.2* is a true loss-of-function allele. For *WRKY17*, we identified one allele from the SALK collection (Alonso et al., 2003), which carries a T-DNA insertion in the 5′ part of the first exon (Figure 2). Q-RT-PCR analysis of the insertion line indicated that no *wrky17* transcript is produced (data not shown). For *WRKY7*, we isolated two alleles, named *wrky7.1* and *wrky7.2* (Figure 2), from the ZIGIA En-insertion population. Both lines carry truncated En-1 transposons, inserted in the first exon of the gene. In both cases, this leads to premature stop codons, resulting in a truncated protein with no WRKY DNA binding domain. In the case of *WRKY15*, we could not identify any insertion mutant in the public mutant collections.

wrky17.1 and *wrky7.1* plants behaved like wild-type plants in both incompatible and compatible interactions with the appropriate *Pst* strains. Bacterial colonization of *wrky17.1* and *wrky7.1* was not significantly different from the colonization of wild-type plants (Figures 3A and 3B), and the disease symptoms observed on mutant leaves after inoculation with virulent *Pst* DC3000 were indistinguishable from those of wild-type controls (Figure 4A for *wrky17.1*; data not shown for *wrky7.1*). On the other hand, both *wrky11* alleles exhibited enhanced resistance to both virulent and avirulent *Pst*. They were less efficiently colonized and, depending on the experiment, 5- to 50-fold less bacteria were present 3 d after inoculation in *wrky11* plants compared with wild-type plants (Figures 3C and 3D). Likewise, in the compatible interaction with
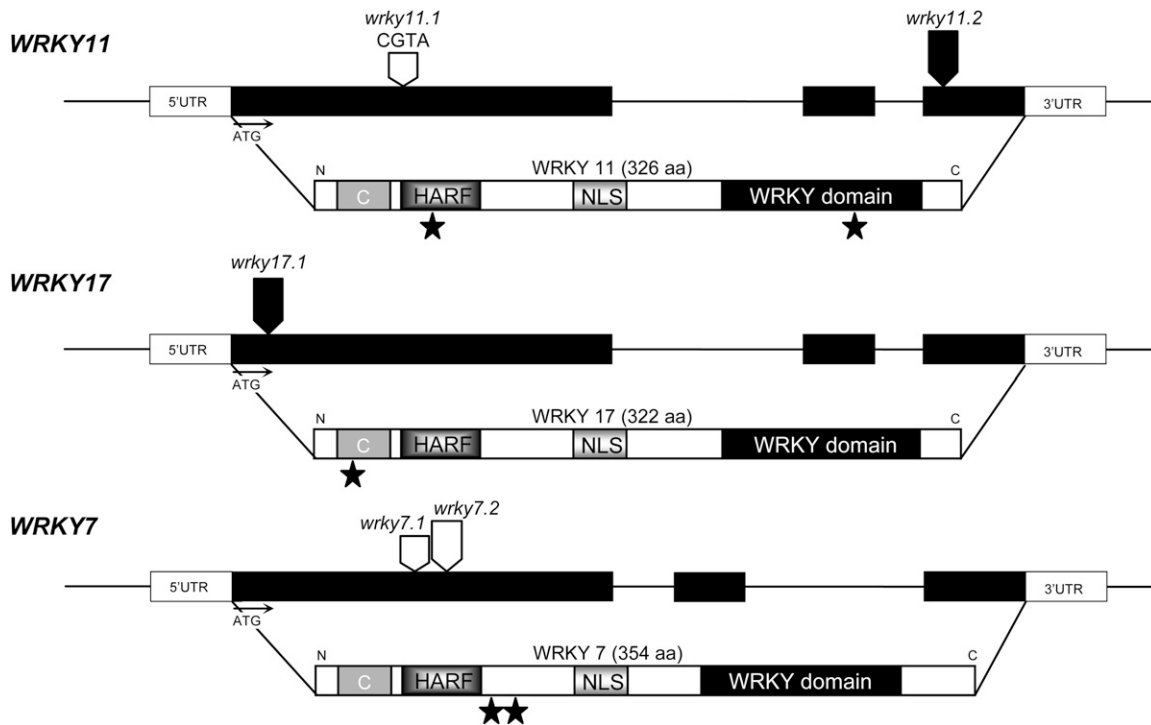
Figure 2. Structures of the *WRKY11*, *WRKY17*, and *WRKY7* Genes, Proteins, and Mutants.

The gene models show the intron/exon structures of the genes, the transcribed (black boxes) and untranscribed regions (UTR; white boxes), and the positions of *En* footprints (open arrows) and T-DNA insertions (closed arrows) in the different mutant alleles. The WRKY11, WRKY17, and WRKY7 proteins are shown with the N-terminal C domain, the HARF domain, the putative nuclear localization sequence (NLS), and the WRKY domain. Black stars indicate the predicted C termini of truncated mutant wrky proteins.

Pst DC3000, we observed weaker disease symptoms on *wrky11* leaves compared with wild-type leaves (Figure 4A for *wrky11.1*; data not shown for *wrky11.2*).

Enhanced resistance is frequently observed in mutants with pleiotropic developmental and/or morphological perturbations and in these cases is associated with the constitutive expression of stress-responsive genes or defense marker genes. No morphological or developmental alterations under standard growth conditions were observed for *wrky11* plants, as indeed for all other WRKY IId mutants tested. Using Q-RT-PCR, we checked the expression of the defense marker genes *PR1*, *PR2*, *PR3*, *PR4*, *PR5*, and *Isochorismate Synthase* (*ICS*) in *wrky11.1* and *wrky11.2* plants and found no altered levels either before or after inoculation (Figure 4D for *PR1* and *PR3*; data not shown for *PR2*, *PR5*, *ICS*, and *wrky11.2*).

Together, these data suggest that WRKY11 is a negative regulator of resistance and that enhanced resistance in *wrky11* mutants is not correlated with constitutive expression or with a stronger or more rapid induction of defense marker genes.

Double Mutant Analysis Reveals That WRKY11 and WRKY17 Act Redundantly as Negative Regulators of Basal Resistance

Analysis of single *wrky* mutant plants did not provide any information concerning the role of *WRKY17* and *WRKY7* in resistance

to *Pst*, although both genes show pathogen-induced expression profiles very similar to *WRKY11*. This was somewhat unexpected, because WRKY17 shows extensive sequence homology with WRKY11 (72% amino acid identity), making it probable that both proteins have similar molecular activities.

To investigate the role of *WRKY17* and *WRKY7* and to evaluate their functional overlap with *WRKY11*, crosses between *wrky11.1* and *wrky17.1* and between *wrky11.1* and *wrky7.2* were performed, and double homozygous plants were isolated from the progeny. F2 lines homozygous for the *wrky11.1* locus and heterozygous for the *wrky17.1* locus were also obtained and used later for cosegregation analysis. The phenotypes of the double mutant plants after inoculation with *Pst* DC3000 and *Pst* DC3000 (*avrRpt2*) were analyzed. As shown in Figure 4A, *wrky11.1 wrky17.1* plants displayed markedly reduced disease symptoms compared with *wrky11.1* in the interaction with *Pst* DC3000, whereas *wrky11.1 wrky7.2* responded similarly to *wrky11.1*. Consistent with these data, bacterial multiplication was reduced significantly in *wrky11.1 wrky17.1* compared with *wrky11.1* and the *wrky11.1 wrky7.2* double mutant (Figure 4B). Three days after inoculation, bacterial density was 5- to 20-fold lower in *wrky11.1 wrky17.1* compared with *wrky11.1* and 20- to 100-fold lower compared with the wild type in both compatible (Figure 4B) and incompatible interactions (see Supplemental Figure 3 online). To confirm the enhanced resistance phenotype of *wrky11.1 wrky17.1*, we also analyzed the progeny of an F2 line
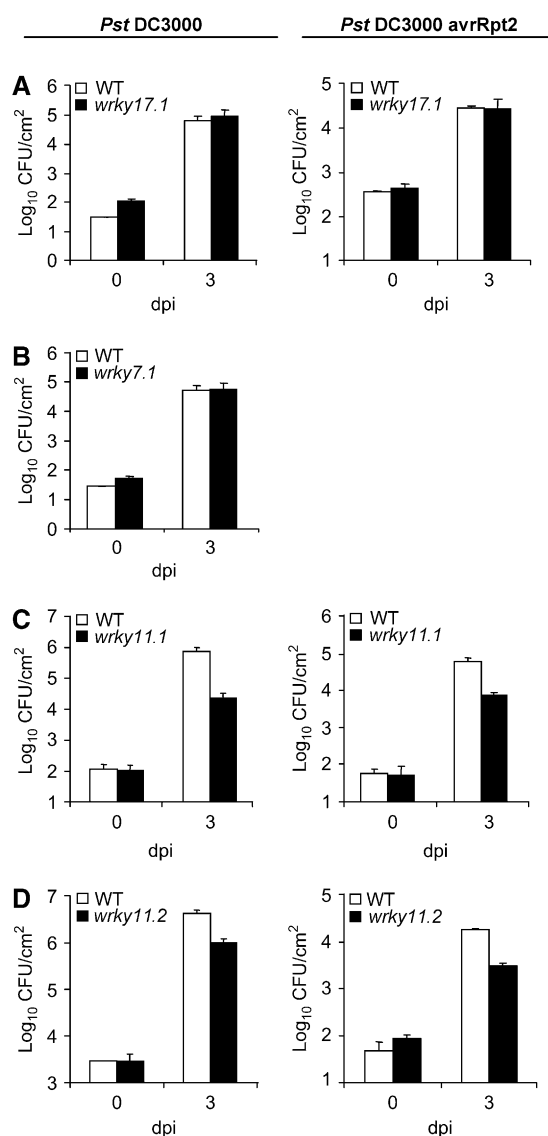
Figure 3. Resistance of *wrky17*, *wrky7*, and *wrky11* Mutants to *Pst* DC3000 and *Pst* DC3000 (*avrRpt2*).

Leaves of 4-week-old wild-type, *wrky17.1* **(A)**, *wrky7.1* **(B)**, *wrky11.1* **(C)**, and *wrky11.2* **(D)** plants were syringe-infiltrated with a bacterial suspension of *Pst* DC3000 (10^5 cfu/mL) and *Pst* DC3000 (*avrRpt2*) (2×10^5 cfu/mL). Mean bacterial densities and SE values calculated from the bacterial densities of six to eight individual plants are shown at 0 and 3 d after inoculation (dpi). These results are representative of three to six independent replications.

that was homozygous for the *wrky11.1* locus and heterozygous for the *wrky17.1* locus. We determined the genotype for the *wrky17.1* locus of 60 individual plants and the corresponding bacterial density 3 d after inoculation with *Pst* DC3000. Again, we found significantly reduced bacterial colonization in mutant plants that were homozygous for the *wrky17.1* locus compared with mutant plants that were heterozygous or wild type (Figure 4C). From this result, we concluded that both WRKY11 and

WRKY17 act as negative regulators of the plant defense response toward *Pst*.

To check whether the enhanced resistance phenotype of *wrky11.1 wrky17.1* plants is correlated with a constitutive or increased activation of defense responses, we analyzed the expression of defense marker genes in the double mutants compared with single mutants and the wild type. No modification in the expression level of the marker genes was detectable in untreated plants. However, during the interaction with *Pst* DC3000, *PR1* and *PR3* were found to be significantly upregulated in the double mutant (Figure 4D).

Screening for Candidate Target Genes by Genome-Wide Expression Analysis in *wrky11.1* and *wrky17.1*

Because both WRKY11 and WRKY17 can be assumed to function as transcription factors involved in the transcriptional reprogramming that occurs after pathogen recognition and contributes to the establishment of resistance, we were particularly interested in identifying their direct or indirect target genes. For this, a genome-wide expression analysis was undertaken using the Arabidopsis ATH1 GeneChip (Affymetrix), which contains 22,500 probe sets representing ~24,000 genes. Three independent experiments were performed in which leaves of wild-type, *wrky11.1*, and *wrky17.1* plants were inoculated with *Pst* DC3000 and harvested at 0, 2, 5, and 12 h after inoculation. Unfortunately, the *wrky11.1 wrky17.1* double mutant was not yet available when this experiment was performed. Each individual sample from each of the three biologically independent experiments was used for the hybridization of one GeneChip. Thus, 36 hybridizations were performed, and expression data for each time point of the three independent experiments were obtained.

As a screen for candidate target genes, we used an analysis with MAS5.0 software (see Methods for details). In this way, 133 genes were identified as differentially expressed in *wrky11.1* and 217 genes were identified as differentially expressed in *wrky17.1* compared with the wild type (see Supplemental Figure 4 online; Supplemental Tables 1 and 2 online list the genes differentially expressed in *wrky11.1* and *wrky17.1*). Only 11 differentially expressed genes were common to both *wrky11.1* and *wrky17.1* mutant lines. Among the genes differentially expressed in *wrky11.1*, we identified at different time points *WRKY11*, which we knew from previous experiments to be weakly but significantly downregulated in *wrky11.1*. This showed that we can indeed identify weakly differentially expressed genes by this method.

Functional Overlap between WRKY11 and WRKY17 in the Regulation of Target Genes Identified by Transcriptome Analysis

To validate some of the candidate genes, and to address the question of a potential functional overlap between WRKY11 and WRKY17, we compared in three independent biological experiments (different from those used for the microarray analysis) the expression of 10 differentially expressed genes in wild-type, *wrky11.1*, *wrky17.1*, and *wrky11.1 wrky17.1* plants. We focused on genes with putative roles in signal perception and signal
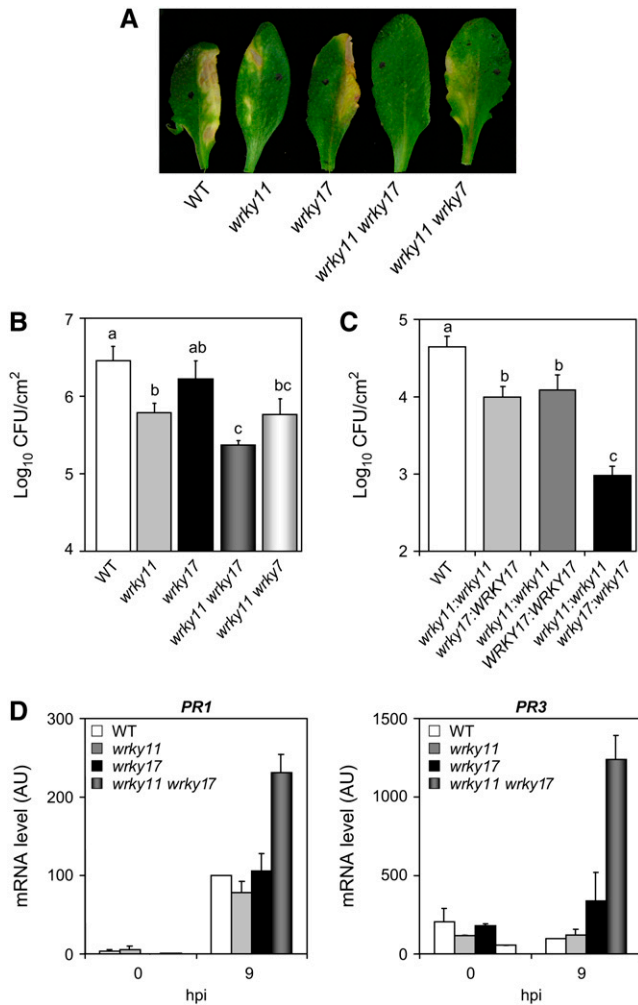
Figure 4. Disease Phenotypes of *wrky11.1* and *wrky17.1* Single Mutant and *wrky11.1 wrky17.1* and *wrky11.1 wrky7.2* Double Mutant Plants.

(A) Representative leaves of wild-type, *wrky11.1*, *wrky17.1*, *wrky11.1 wrky17.1*, and *wrky11.1 wrky7.2* plants 6 d after syringe inoculation with a bacterial suspension of *Pst* DC3000 at a density of 2×10^5 cfu/mL.

(B) Growth of *Pst* DC3000 in wild-type, *wrky11.1*, *wrky17.1*, *wrky11.1 wrky17.1*, and *wrky11.1 wrky7.2* plants. Inoculation was performed with a bacterial suspension of 10^5 cfu/mL, and bacterial growth was determined at 3 d after inoculation. Mean bacterial densities and SE values calculated from six to eight replicate plants are shown. According to Student's *t* test (P ≤ 0.05), means of cfu do not differ significantly if they are indicated with the same lowercase letter. These results are representative of four independent experiments.

(C) Cosegregation analysis using the progeny of a *wrky11.1:wrky11.1 wrky17.1:WRKY17* plant. Wild-type plants and 60 plants from the progeny of an F2 line homozygous for the *wrky11.1* mutation and heterozygous for the *wrky17.1* mutation were infiltrated with a bacterial suspension of 10^5 cfu/mL. Bacterial growth was determined at 3 d after inoculation. In parallel, the plants were genotyped individually for the *wrky17.1* mutation. The mean bacterial densities and SE values were calculated from at least 14 replicate plants. According to Student's *t* test (P ≤ 0.05), means of cfu do not differ significantly if they are indicated with the same lowercase letter.

(D) Expression patterns of the defense-related genes *PR1* and *PR3* in *wrky11.1* and *wrky17.1* single and double mutants. The transcript levels

transduction, such as *FRK1/SIRK* (Asai et al., 2002; Robatzek and Somssich, 2002), *RFO1* (Diener and Ausubel, 2005), *CRK5* (Du and Chen, 2000; Chen et al., 2003), *ATKC1* (Reintanz et al., 2002), and *RRPK* (for *Related to Receptor Protein Kinases*), because these classes of genes are overrepresented in the candidate gene lists (see Supplemental Table 3 online). In noninoculated *wrky11.1* plants, a large number of resistance gene homologs (5 of 30 genes) and receptor-like protein kinases (5 of 30 genes) seem to be upregulated. A high enrichment for genes encoding protein kinases with putative functions in signal perception or signal transduction was observed among the genes upregulated in *wrky11.1* at 5 h after inoculation (8 of 44 genes; see Supplemental Table 3A online) and in *wrky17.1* at 2 h after inoculation (18 of 110 genes; see Supplemental Table 3C online). In addition, two JA-associated genes, *LOX2* and *AOS*, were analyzed, because a high proportion of genes downregulated in *wrky11.1* at 5 h (see Supplemental Table 3B online) have been described in the literature as being JA-responsive, including *VSP1*, *VSP2*, and *COR1* (Benedetti et al., 1995, 1998; Tsuchiya et al., 1999), or are strongly JA-inducible according to transcriptome databases (see Supplemental Figure 5 online). Three of the genes, *LOX2*, *AOS*, and *JMT*, encode enzymes of JA biosynthesis or derivatization (Bell et al., 1995; Laudert et al., 1996; Seo et al., 2001). Finally, the expression of *WRKY11* and two other *WRKY* genes with described functions in the regulation of plant defense, *WRKY54* (Kalde et al., 2003) and *WRKY70* (Li et al., 2004), was analyzed.

All of the genes analyzed showed significantly altered expression in the *wrky* single and/or double mutants by Q-RT-PCR analysis (Figure 5), confirming the trends observed in the transcriptome analysis. Genes upregulated or downregulated in the microarray experiment could be confirmed by Q-RT-PCR as, respectively, upregulated or downregulated. In some cases, however, differential expression was not observed in the corresponding single mutant but only in the double mutant (*WRKY70*) or at a different time point (*LOX2* and *AOS*). These differences may be attributable to variations in the extent of functional complementation between the two WRKY homologs or to variations in the kinetics of gene induction. According to their expression profiles in the different mutants, the validated target genes could be classified into three groups. Group 1, the largest one, is characterized by a strong upregulation in inoculated *wrky11.1* plants. This group contains *ATKC1*, *FRK1/SIRK*, *RFO1*, *CRK5*, and *RRPK* (Figure 5). The expression of these genes in *wrky17.1* and *wrky11.1 wrky17.1* plants is either not altered or only increased slightly compared with that in wild-type plants but is reduced significantly compared with that in *wrky11.1* plants. These results suggest that WRKY17 acts in the same pathway as

of *PR1* and *PR3* were determined by Q-RT-PCR with cDNA generated from leaves of 4-week-old plants inoculated with *Pst* DC3000 at 10^7 cfu/mL. The expression values of the individual genes were normalized using the expression level of β-*Tubulin4* as an internal standard and are expressed as percentages with respect to the value of wild-type plants at 9 h after inoculation (hpi), which is set at 100%. Mean expression values and SE values were calculated from the results of three independent experiments.
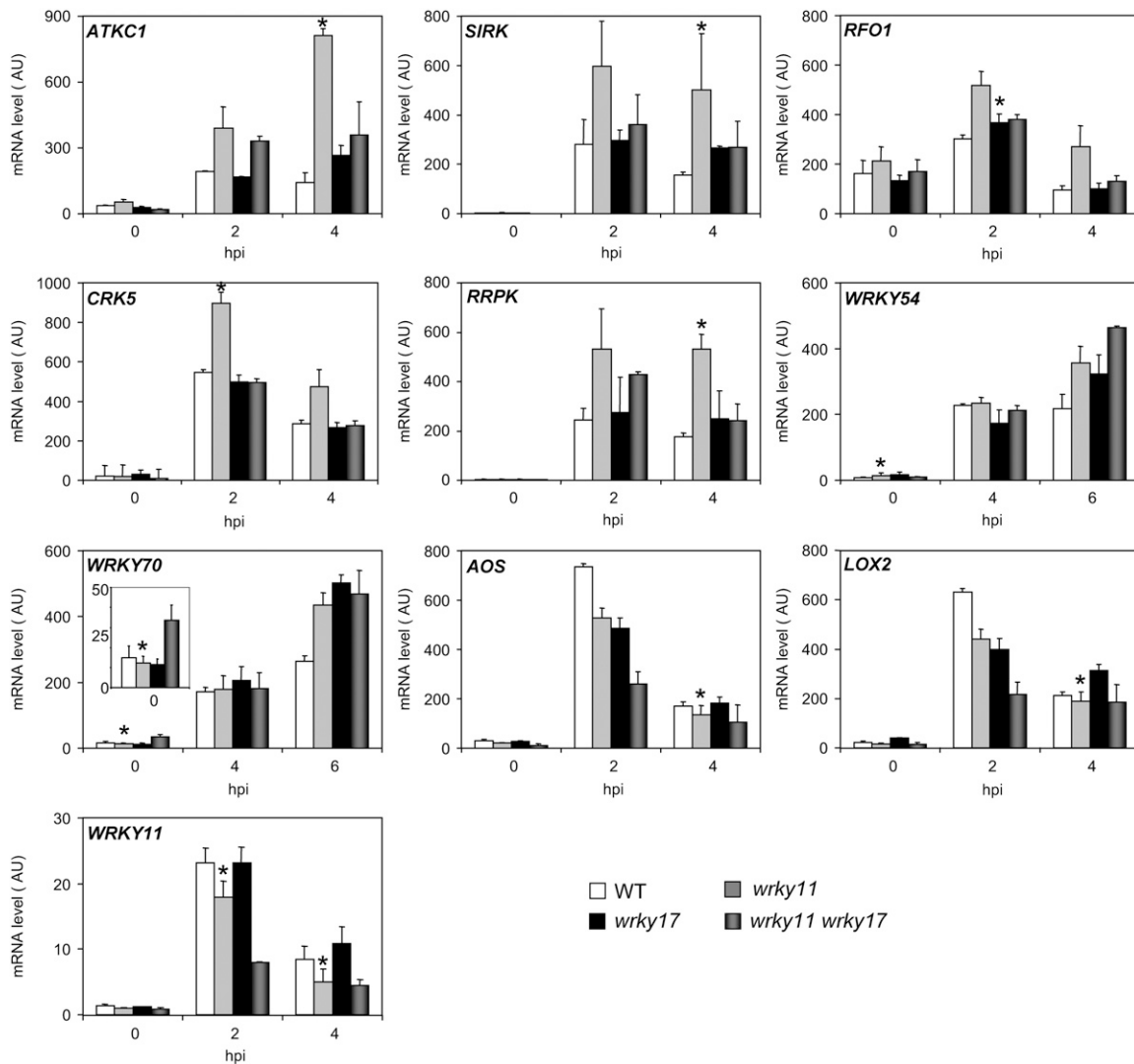
Figure 5. Expression Analysis of Genes Potentially Regulated by *WRKY11* and/or *WRKY17*.

Transcript levels of *ATKC1*, *FRK1/SIRK*, *RFO1*, *CRK5*, *RRPK*, *WRKY54*, *WRKY70*, *LOX2*, *AOS*, and *WRKY11* were determined by Q-RT-PCR with cDNA generated from leaves of 4-week-old plants inoculated with *Pst* DC3000 at 10^7 cfu/mL. The expression values of the individual genes were normalized using the expression level of β-*Tubulin4* as an internal standard. Mean expression values and SE values were calculated from the results of three independent experiments. Stars indicate in which mutant and at which time point genes were identified as differentially expressed in the microarray analysis. hpi, h after inoculation.

WRKY11 in the regulation of this group of genes, downstream of WRKY11 and in an opposite manner, because the loss of *WRKY17* abolishes the loss-of-function effect of *WRKY11*. It also indicates that WRKY11 and WRKY17 have specific nonredundant functions in the regulation of these genes.

The second group consists of *WRKY54* and *WRKY70* and is characterized by similar upregulation in *wrky11.1*, *wrky17.1*, and *wrky11.1 wrky17.1* at 6 h after inoculation and by upregulation in untreated *wrky11.1 wrky17.1* for *WRKY70* (Figure 5). The synergistic effect of losing *WRKY11* and *WRKY17* function in noninfected plants suggests that they act in a partially redundant manner as negative regulators of *WRKY70*. Finally, the third

group contains *WRKY11* and the two JA-responsive genes *AOS* and *LOX2*. In *wrky11.1* and *wrky17.1* single mutants, the expression of these genes is either not altered or weakly but significantly downregulated (Figure 5), whereas in the *wrky11.1 wrky17.1* double mutant, their expression is strongly downregulated. This finding suggests that WRKY11 and WRKY17 positively regulate the expression of this group of genes with partial redundancy.

Because WRKY17 regulates *WRKY11* expression, we asked whether additional cross-regulation exists among the IId subfamily members. Therefore, the expression of *WRKY17*, *WRKY7*, and *WRKY15* was analyzed in *wrky11.1*, *wrky11.2*, *wrky17.1*, and *wrky11.1 wrky17.1* mutants after inoculation with *Pst* DC3000

and *Pst* DC3000 (*avrRpt2*). Although *WRKY7* and *WRKY15* expression was not altered in any of the mutant plants (data not shown), *WRKY17* was substantially upregulated in the *wrky11.1* and *wrky11.2* mutants compared with wild-type plants (Figure 6), indicating that WRKY11 negatively regulates the expression of *WRKY17*. Whether WRKY17 regulates its own expression could not be tested because of the lack of detectable *WRKY17* transcripts in the *wrky17.1* T-DNA insertion line.

DISCUSSION

Using mutant analysis, we were able to demonstrate that WRKY11 and WRKY17 act as negative regulators of basal resistance during compatible and incompatible interactions with *Pst*. This lends strong genetic support to the hypothesis that members of this gene family play important roles in the regulation of plant defense. The enhanced resistance conferred

Figure 6. *WRKY17* Expression Analysis in Wild-Type and Mutant Plants.

The transcript level of *WRKY17* was determined by Q-RT-PCR with cDNA generated from leaves of 4-week-old wild-type (closed diamonds), *wrky11.1* (closed squares), and *wrky11.2* (open squares) plants inoculated with *Pst* DC3000 or *Pst* DC3000 (*avrRpt2*) at 10^7 cfu/mL. The expression values of the individual genes were normalized using the expression level of β-*Tubulin4* as an internal standard. These results are representative of four independent replicates. hpi, h after inoculation.

by the loss of WRKY11 and WRKY17 function reveals a certain specificity, because resistance to avirulent and virulent strains of *X. campestris* pv *campestris* is not altered in *wrky11* single and *wrky11 wrky17* double mutants (N. Journot-Catalino and T. Kroj, unpublished data). In addition, transcriptome analysis and expression studies of selected defense genes indicate that this is not attributable to constitutive expression of defense responses. Although we have been able to correlate increased resistance with the altered expression of a certain number of genes, we cannot as yet establish the precise relationship between the altered expression of these genes and enhanced resistance. Nevertheless, we have identified specific and redundant functions of WRKY11 and WRKY17 in the regulation of plant resistance, both during normal growth and upon pathogen challenge.

The Biological Significance of the Negative Regulation of Resistance by WRKY11 and WRKY17

At first sight, it may appear surprising that a negative regulator of defense responses is rapidly induced during the establishment of resistance. However, it must be borne in mind that the plant defense system can be deleterious to the host, as shown in mutants with constitutively activated defense responses or with hypersensitive response lesion–mimic phenotypes, which often show stunted growth and low fertility (Lorrain et al., 2003). In addition, the activation of defense and even the maintenance of surveillance by the expression of *R* genes is associated with significant costs for the plant (Heil, 2002; Brown, 2003). For these reasons, the plant defense system needs to be under tight, fine-tuned regulation to be efficient but also beneficial to the plant. Our study suggests that the biological function of WRKY11 and WRKY17 could be both to limit the expression of the pathogen surveillance system in nonstimulated plants and to attenuate the expression of plant defense responses upon challenge with pathogens, thus contributing to a balanced allocation of resources by limiting the activation of defense-related functions.

Another important role of such negative regulators of defense responses could be to ensure an equilibrated defense response, which is effective not only against *Pst* but also against the panoply of other potentially pathogenic microorganisms (Thomma et al., 2001). It has repeatedly been observed that enhanced resistance to one class of pathogens results in enhanced susceptibility to another class and that in many of these cases the mutually antagonistic action of SA and JA is disturbed (Kloek et al., 2001; Veronese et al., 2006). In favor of this idea, *wrky11* plants show enhanced susceptibility to virulent strains of *Ralstonia solanacearum* (Y. Marco and X. Barlet, personal communication). Therefore, it will be interesting in the future to test the resistance and susceptibility of the different single and multiple *wrky11*, *wrky17*, and *wrky7* mutants to a wider range of pathogens with varying physiologies and invasion strategies.

Redundant versus Specific Functions of WRKY11 and WRKY17

A frequent problem in the study of plant transcription factors is functional overlap between closely related homologs, attributable to similar molecular activities and to overlapping expression
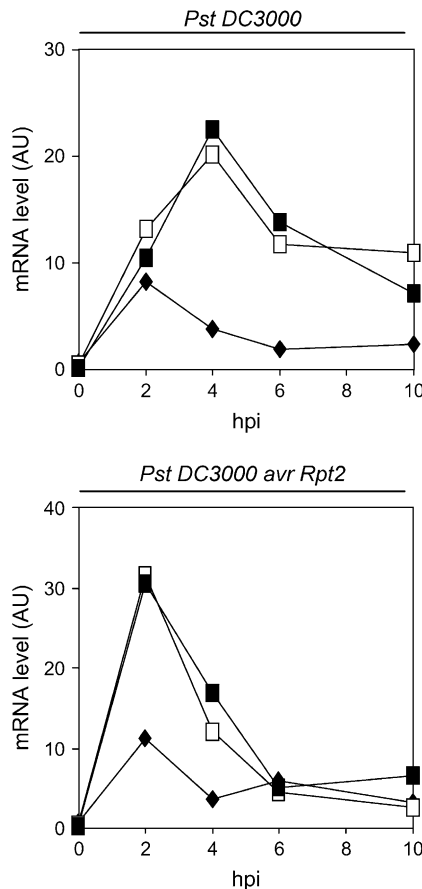
patterns. For this reason, knockouts in multiple transcription factors have often been necessary to produce informative phenotypes (Liljegren et al., 2000; Kroj et al., 2003; Zhang et al., 2003; Xu et al. 2006). We have also found strong evidence for functional overlap between WRKY11 and WRKY17. The resistance phenotype of the double mutant is not simply the sum of the phenotypes of the single mutants but reflects a synergistic interaction between mutations in *WRKY11* and *WRKY17*. Analyzing the expression of single genes and of the entire genome indicated that no target genes are strongly deregulated in *wrky11* or *wrky17* and revealed only subtle changes in the expression levels of individual genes. This may also be attributable to partial compensation of one factor by its closest homolog.

However, our data also indicate specific functions for the two WRKY proteins. First, *wrky11* single mutants show an enhanced resistance phenotype; second, the group 1 genes are upregulated in *wrky11* in a *WRKY17*-dependent manner. Because *WRKY17* is also upregulated in *wrky11* mutant plants, the regulation of group 1 genes can be explained by a model in which WRKY11 and WRKY17 act in a sequential and partly nonoverlapping manner (Figure 7A).

A molecular view of specificity and redundancy in WRKY protein function has emerged from a recent study describing the elicitor-responsive binding of the parsley (*Petroselinum crispum*) WRKY1 protein to its own promoter and to the promoter of the defense gene *Pc PR1-1* in vivo (Turck et al., 2004). Regulatory W-box elements seem to be constantly occupied by WRKY proteins, but this occupancy is changing dynamically in a stimulus-dependent manner. Different WRKY factors are proposed to compete with one another for individual WRKY binding sites, generating a dynamic equilibrium of W-box occupancy. This equilibrium seems to be regulated by posttranslational modifications of individual WRKY proteins and their de novo synthesis or degradation (Turck et al., 2004). In this conceptual framework, loss of function of one individual WRKY protein may lead to a shift in equilibrium, with the respective WRKY protein being replaced by certain homologs at various W-boxes sites. Depending on the *trans*-activating or repressing activity of these homologs, the transcriptional output of individual downstream target genes may be either positively or negatively affected.

To better understand the specific and redundant functions of WRKY11 and WRKY17, it is indispensable to identify direct target genes of the two factors. We found a significant overrepresentation of W-boxes in the promoters of candidate genes upregulated in *wrky11* plants (P < 10^{-5} in the −1.5-kb region according to an analysis with ATHENA [O'Connor et al., 2005]), suggesting that several of them may be regulated directly by WRKY11 and/or WRKY17. For certain genes, such as *FRK1/SIRK*, additional evidence exists indicating direct regulation by WRKY proteins binding to defined W-boxes within their promoters (Asai et al., 2002; Robatzek and Somssich, 2002). In vitro binding studies and promoter analysis are a prerequisite to identify direct target genes of WRKY11 and WRKY17. By this means, WRKY11 binding to several of the W-boxes in the *FRK1/SIRK* promoter has been demonstrated (I. Ciolkowski and I. Somssich, unpublished data). Ultimately, however, defining in vivo target genes using methods such as chromatin immunoprecipitation (Orlando, 2000; Hanlon and Lieb, 2004) will be essential to decipher the dynamics of
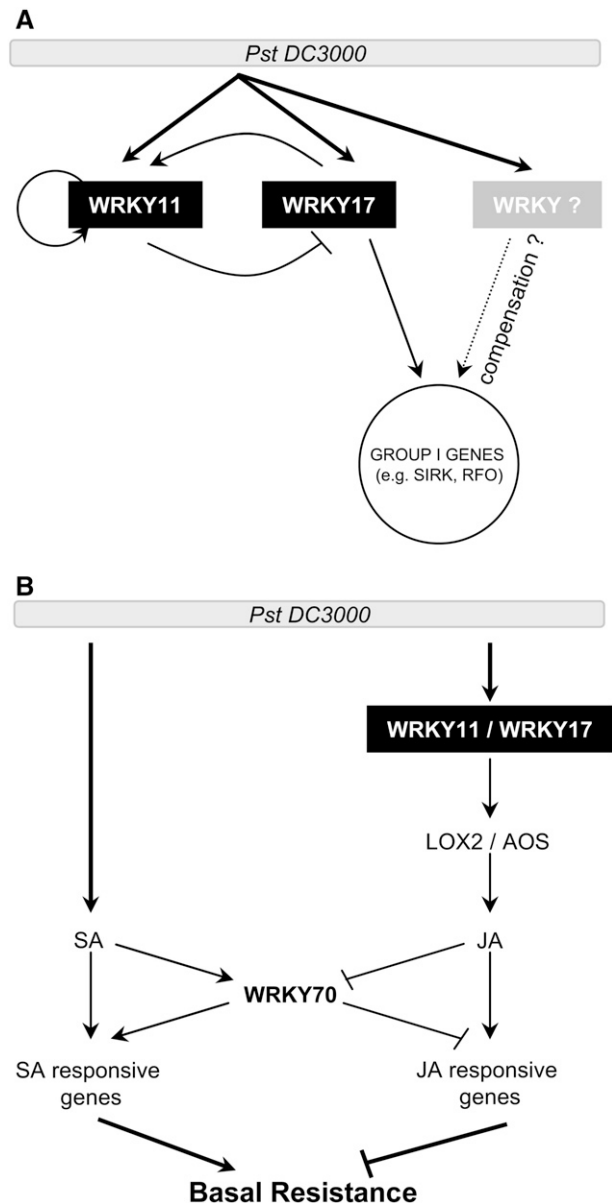

Figure 7. Working Model for WRKY11 and WRKY17 Action.

(A) Group 1 genes are regulated in a positive manner by WRKY17, which is itself negatively regulated by WRKY11. In *wrky11*, WRKY17 is upregulated, leading to stronger expression of the downstream genes. In *wrky17* and *wrky11 wrky17* plants, WRKY17 function may be replaced by another functionally similar WRKY factor that is independent of WRKY11. This leads to an expression level of group 1 genes that is unaltered or increased slightly compared with that in wild-type plants.

(B) WRKY11 and WRKY17 regulate in a partially redundant manner the *Pst*-induced expression of the central JA biosynthetic enzymes *LOX2* and *AOS* and by this may indirectly modulate the level of pathogen-responsive JA production. In addition, they negatively regulate the expression of *WRKY70*, which is under the control of JA and acts as an integrator of the mutually antagonistic JA and SA pathways (Li et al., 2004). By these two interconnected mechanisms, WRKY11 and WRKY17 may induce the expression of JA-responsive genes, repress the expression of genes responsive to SA, and ultimately negatively regulate basal resistance.

WRKY protein–DNA interactions under physiological conditions at individual promoter sites and to define the extent to which WRKY11 and WRKY17 directly act as transcriptional repressors or activators of target genes on a genome-wide basis.

Function of WRKY11 and WRKY17 in the Regulation of Defense Responses

In *Pst*-inoculated plants, WRKY11 attenuates the induction of a range of pathogen-responsive genes, notably protein kinases with putative functions in signal perception and signal transduction. Protein kinases from different classes play important roles in the activation of plant defense (Nürnberger and Scheel, 2001; Romeis, 2001), and their overexpression leads to enhanced resistance (Chen et al., 2003). Thus, enhanced resistance in *wrky11* and *wrky11 wrky17* could in part result from the amplification of signal transduction attributable to the upregulation of signaling elements.

In addition to their function as negative regulators, WRKY11 and WRKY17 act also as positive regulators of gene expression. Among the genes downregulated in *wrky11* after pathogen challenge, JA-responsive genes are particularly abundant, suggesting that WRKY11 and maybe also WRKY17 positively modulates JA signaling, either upstream of JA production or downstream of JA. Expression analysis of two key enzymes of JA biosynthesis, *LOX2* and *AOS*, in *wrky11* and *wrky17* single and double mutant plants showed that both are regulated positively and redundantly by WRKY11 and WRKY17. LOX2 has been demonstrated to be essential for JA production in the wound response (Bell et al., 1995) and may have a similar function in pathogen responses. AOS, which catalyzes the first step of the octadecanoid pathway leading to jasmonates, has been proposed to be a key regulatory element in the production of jasmonates (Laudert et al., 1996; Laudert and Weiler, 1998; Park et al., 2002). Together with the fact that the expression of *WRKY11* and *WRKY17* is not affected by JA (transcriptome database at Genevestigator [Zimmermann et al., 2004]; see Supplemental Figure 4 online), these results imply a model in which WRKY11 and WRKY17 act upstream of JA by positively regulating the *Pst*-stimulated accumulation of jasmonates (Figure 7B).

The timing of *WRKY11* and *WRKY17* expression is consistent with the timing of the induction of the JA biosynthetic enzymes *AOS* and *LOX2* and with the timing of JA accumulation, which occurs within the first hour of the interaction with *Pst* (De Vos et al., 2005). This model can explain the enhanced-resistance phenotype of *wrky11* and *wrky11 wrky17* and the upregulation of the SA-dependent marker gene *PR1* in the double mutant, because JA negatively influences SA action and basal resistance to *Pst* (Kloek et al., 2001; Glazebrook et al., 2003; Devoto et al., 2005). Another link between WRKY11 and WRKY17 action and JA signaling is the negative regulation of *WRKY70* by both proteins. WRKY70 has been shown to act as a negative regulator of the expression of JA-responsive genes downstream of JA and as a positive regulator of SA-induced genes such as *PR1* (Li et al., 2004, 2006). Because the promoter of *WRKY70* contains no W-boxes, its expression is presumably not regulated directly by WRKY proteins. However, JA has been shown to negatively regulate *WRKY70* transcript levels, whereas SA regulates

WRKY70 transcription positively (Li et al., 2004). Upregulation of *WRKY70* in *wrky11* and *wrky17* single and double mutants could thus result from reduced JA levels and contribute to the downregulation of JA-responsive genes. Future experiments will be required to validate the roles of WRKY11 and WRKY17 in positively regulating the JA pathway. In particular, determining the concentrations of JA and other jasmonates in the wild type, *wrky11* and *wrky17* single mutants, and the *wrky11 wrky17* double mutant will be of particular interest.

WRKY11 and WRKY17 positively regulate *WRKY11* expression in a partly redundant manner, whereas WRKY11 negatively regulates the expression of *WRKY17* (Figure 7A). As the promoters of both genes are highly enriched for W-boxes (Dong et al., 2003), it is conceivable that direct WRKY interactions are involved. The complex pattern of autoregulation and cross-regulation observed in the IId subfamily seems to be a general characteristic of the WRKY family. For example, *WRKY6* has been demonstrated to negatively regulate its own expression and the expression of the closely related gene *WRKY42* (Robatzek and Somssich, 2002), whereas the group III WRKY protein WRKY54 has been shown to influence in a complex manner the expression of other group III *WRKY* genes in response to different pathogens and pathogen-related treatments (Kalde et al., 2003). In addition, cross-regulation between distantly related WRKY genes also exists, as exemplified in this study, which shows that WRKY11 and WRKY17 negatively regulate the expression of *WRKY70* and *WRKY54*. The emerging picture appears to be that of a highly interconnected network of WRKY transcription factors modulating *WRKY* gene expression and thereby fine-tuning the plant defense system.

In summary, we have been able to demonstrate a particular function for individual WRKY genes in the regulation of plant defense. Apart from the role that our study establishes for WRKY11 and WRKY17 in the negative regulation of basal resistance, we show that specific functions can be attributed to individual WRKY proteins. However, this study has also revealed the existence of considerable functional overlap between closely related WRKY transcription factors and, as a consequence, the subtle and quantitative nature of single mutant phenotypes. Finally, it illustrates the existence of a network of WRKY transcription factors that regulate and integrate signaling through the antagonistic SA and JA signal transduction pathways.

METHODS

Plant Materials and Growth Conditions

All *Arabidopsis thaliana* lines used in this study are in the Columbia background. As a wild-type control, we used Col-0 (Nottingham Arabidopsis Stock Centre [NASC] accession number N1093). Plants were grown in Jiffy pods in a growth chamber at 22°C, with a 9-h light period and a light intensity of 190 μmol·m^{-2}·s^{-1}. All experiments were performed with 4- to 5-week-old plants.

Bacterial Strains, Plant Inoculation Procedures, and Bacteria Growth Measurements

Pseudomonas syringae pv *tomato* (*Pst*) strains were grown at 29°C on King B's medium supplemented with 50 μg/mL rifampicin for *Pst* DC3000

and 50 μg/mL rifampicin and 10 μg/mL tetracycline for *Pst* DC3000 (*avrRpt2*) (Whalen et al., 1991). Plant inoculations and in planta bacterial growth analysis were performed essentially as described previously (Lorrain et al., 2004) using six to eight replicate plants per experiment. For the determination of disease symptoms and in planta bacterial growth, we used an inoculum of 10^5 cfu/mL for *Pst* DC3000 and of 2×10^5 cfu/mL for *Pst* DC3000 (*avrRpt2*). For gene expression analysis, we used an inoculum of 10^7 cfu/mL.

Identification of Insertion Mutants and Generation of Double Mutants

The mutant lines *wrky7.1*, *wrky7.2*, and *wrky11.1* were derived from insertion mutants identified by a PCR-based screen of an *En-1* insertion population (Wisman et al., 1998). The use of gene-specific and *En-1*–specific primers led to the identification of the line 5AAH57 with an *En-1* insertion in *WRKY11* and the lines 5AAO102 and 6AAL50 with *En-1* insertions in *WRKY7*. The *En-1* insertions were confirmed by DNA gel blot analysis, and the insertion sites were determined by sequencing. Footprint mutants were identified with gene-specific primers flanking the original *En-1* insertion site. The individual lines 6AAL50.3 and 5AAO102.12, which contain fragments of the original *En-1* transposon, and 5AAH57.3, which carries a 4-bp insertion, were backcrossed at least five times to eliminate additional *En-1* insertions, leading to the isolation of the *wrky7.1*, *wrky7.2*, and *wrky11.1* lines.

The *wrky11.2* and *wrky17.1* mutant lines were derived by backcrossing from the T-DNA insertion lines GABI-KAT 184D06 (Li et al., 2003) and SALK_176337 (Alonso et al., 2003), respectively. The position of the insertions was confirmed by PCR and sequencing, using the primers 5′-GGAGCCGGAGTTGTCACTTT-3′ (gene-specific) and 5′-CCCTTTAG-GGTTCCGATTTAGTGCT-3′ (left border of the T-DNA) for *wrky17.1* and the primers 5′-CCACCGTCTAGTGTAACACTCGAT-3′ (gene-specific) and 5′-GGGCTACACTGAATTGGTAGCTC-3′ (left border of the T-DNA) for *wrky11.2*.

The double mutant lines *wrky11.1 wrky17.1* and *wrky11.1 wrky7.1* were generated by crossing backcrossed homozygote mutant lines and determining the genotype of plants from the progeny by PCR analysis. The 4-bp footprint in *wrky11.1* generates a new *Rsa*1 restriction site that allowed the generation of a cleaved-amplified polymorphic sequence marker (primers 5′-GGAGAAACTCTCTGAGTGCTCAT-3′ and 5′-GCT-CCGAGATCACTGACTT-3′; digest of the 482-bp fragment by *Rsa*1).

Phylogenetic Analysis

Sequence alignment was performed with ClustalW and manually adjusted to get maximal alignment for the shared C domain, the putative nuclear localization signal, and the WRKY domain (Eulgem, 2000). Phylogenetic analysis was performed with the MEGA3 software package using neighbor-joining, UPGMA, maximum-parsimony, or minimum-evolution methods with 1000 bootstrap trials and complete gap deletion.

RNA Extraction and Q-RT-PCR Analysis

Material for RNA analysis was ground in liquid nitrogen, and total RNA was isolated using the Nucleospin RNA plant kit (Macherey-Nagel) according to the manufacturer's recommendations. Reverse transcription was performed using 1 μg of total RNA and SuperScript reverse transcriptase II (Invitrogen). Quantitative PCR was run on a Lightcycler system (Roche Diagnostics) according to the manufacturer's recommendations with the following conditions: 1 cycle of 9 min at 95℃, and 45 cycles of 5 s at 95℃, 10 s at 65℃, and 20 s at 72℃. β-*Tubulin4* was used as an internal standard. The primer sets used in the different experiments are listed in Supplemental Table 4 online. The specificity of the amplifi-

cations was verified by analysis of the PCR products on agarose gels to ensure that only a single band was present and by melting curve analysis at the end of each experiment. Efficiency of the amplification was verified by the analysis of standard curves.

Microarray Experiments and Data Analysis

Samples were harvested and RNA was prepared as described for Q-RT-PCR analysis. To verify the induction of basal defense responses, the expression of the genes *PR1*, *ICS*, and *WRKY11* was analyzed in the different experiments by Q-RT-PCR. Despite variations in kinetics and the amplitude of induction, expression patterns for the tested genes in the three experiments were very similar to one another and corresponded to the patterns observed in previous experiments. Processing of RNA, ATH1 GeneChip (Affymetrix) hybridization, and raw data collection were performed as described (Redman et al., 2004).

Using MAS5.0 statistical algorithms (Affymetrix; http://www.affymetrix.com/support/technical/whitepapers/sadd_whitepaper.pdf), GeneChip fluorescence intensity data were used to calculate for each probe set signal intensities (scaled to an average signal intensity of 100) and signal log ratios for pairwise slide-to-slide comparisons. In addition, MAS5.0 nonparametric rank tests (Liu et al., 2002) were used to determine whether a gene was detectable (detection call a for absent or p for present) and whether the signal log ratios represented a genuine change in mRNA level (change call i for increased, d for decreased, or nc for no change). Signal log ratios and change calls were determined for each mutant sample compared with its corresponding wild-type sample from the same biological experiment and the same time point.

Probe sets had to meet two criteria to be selected for further analysis. Those that were considered upregulated in a mutant at a given time point had to be present in the corresponding three replicate mutant samples (detection call p with an associated P < 0.05) and induced in all three independent biological experiments (change call i with an associated P < 0.006). Those that were considered downregulated in a mutant at a given time point had to be present in the corresponding three replicate wild-type samples (detection call p with an associated P < 0.05) and decreased in all three independent biological experiments (change call d with an associated P > 0.994).

To analyze whether after inoculation with *Pst* genes were upregulated or downregulated in wild-type plants (see Supplemental Tables 1 and 2 online), transcriptome data were analyzed by an alternative approach using Bioconductor packages (Gentlemen et al., 2005). Intensities were calculated from raw data by the robust multiple-array average expression measure (Irizarry et al., 2003). Subsequently, for time points 2, 5, and 12 h, a pairwise comparison between the intensities of the three replicates of each time point and the three replicates of time 0 was performed to identify significantly differentially expressed genes. This analysis was performed with the LIMMA package using an empirical Bayes linear modeling approach (Smyth, 2004, 2005) and by correcting obtained P values for multiple testing according to Benjamini and Hochberg (1995).

For functional classification of the differentially expressed genes, GO annotations at The Arabidopsis Information Resource (Berardini et al., 2004; Harris et al., 2004), FUN-CAT annotations at the Munich Information Center for Protein Sequences (Ruepp et al., 2004), and MapMan version 1.6.0 (Thimm et al., 2004) were used. Analysis of promoter regions was performed with ATHENA (O'Connor et al., 2005) and AGRIS (Davuluri et al., 2003; Palaniswamy et al., 2006). Public transcriptome data were analyzed using Genevestigator (Zimmermann et al., 2004).

Accession Numbers

Locus identifiers of the genes from this article are as follows: At4g24240 (*WRKY7*), At2g23320 (*WRKY15*), At4g31550 (*WRKY11*), At2g24570 (*WRKY17*), At2g30590 (*WRKY21*), At3g04670 (*WRKY39*), At5g28650

(*WRKY74*), At5g44340 (β-*Tubulin4*), At2g14610 (*PR1*), At3g57260 (*PR2*), At3g12500 (*PR3*), At3g04720 (*PR4*), At1g75040 (*PR5*), At1g74710 (*ICS*), At2g19190 (*FRK1/SIRK*), At2g40750 (*WRKY54*), At3g56400 (*WRKY70*), At4g23130 (*CRK5*), At1g79670 (*RFO1*), At3g45140 (*LOX2*), At5g42650 (*AOS*), At4g32650 (*ATKC1*), and At3g46280 (*RRPK*). Microarray data have been deposited in the NASCArrays database under the experiment reference number NASCARRAYS-393.

Supplemental Data

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

Supplemental Table 1. Genes Differentially Expressed in *wrky11.1* Plants.

Supplemental Table 2. Genes Differentially Expressed in *wrky17.1* Plants.

Supplemental Table 3. Selected Genes Differentially Expressed in *wrky11* and *wrky17*.

Supplemental Table 4. Primers Used for Q-RT-PCR.

Supplemental Figure 1. Alignment of the WRKY IId Subfamily Proteins.

Supplemental Figure 2. Expression Pattern of *WRKY11* and *WRKY17* in Response to Abiotic Stresses.

Supplemental Figure 3. *WRKY11* Expression in *wrky11.1*.

Supplemental Figure 4. Resistance of *wrky11.1* and *wrky17.1* Single and Double Mutants to *Pst* DC3000 (*avrRpt2*).

Supplemental Figure 5. Venn Diagrams of Genes Upregulated or Downregulated in *wrky11* and *wrky17*.

Supplemental Figure 6. JA Responsiveness of Genes Downregulated in *wrky11* at 5 h after Inoculation.

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