The *Arabidopsis* ATAF1, a NAC Transcription Factor, Is a Negative Regulator of Defense Responses Against Necrotrophic Fungal and Bacterial Pathogens

Xiao'e Wang,¹ B. M. Vindhya S. Basnayake,¹ Huijuan Zhang,¹ Guojun Li,¹ Wei Li,¹ Nasar Virk,¹ Tesfaye Mengiste,² and Fengming Song¹

¹State Key Laboratory for Rice Biology, Institute of Biotechnology, Zhejiang University-Huajiachi Campus, Hangzhou, Zhejiang 310029, People's Republic of China; ²Department of Botany and Plant Pathology, Purdue University, West Lafayette, IN 47907, U.S.A.

Submitted 11 February 2009. Accepted 10 June 2009.

Transcription factors of the NAC family are known to be involved in various growth or developmental processes and in regulation of response to environmental stresses. In the present study, we report that *Arabidopsis* ATAF1 is a negative regulator of defense responses against both necrotrophic fungal and bacterial pathogens. Expression of *ATAF1* was downregulated after infection with *Botrytis cinerea* or *Pseudomonas syringae* pv. *tomato* or after treatment with salicylic acid (SA), jasmonic acid, and 1-amino cyclopropane-1-carboxylic acid (the precursor of ethylene biosynthesis). Transgenic plants that overexpress the *ATAF1* gene (*ATAF1*-OE) showed increased susceptibility while expression of an *ATAF1* chimeric repressor construct (*ATAF1*-SRDX) exhibited enhanced resistance to *P. syringae* pv. *tomato* DC3000, *B. cinerea*, and *Alternaria brassicicola*. The *ataf1* mutant plants showed no significant resistance against the pathogens tested. After inoculation with *B. cinerea* or *P. syringae* pv. *tomato* DC3000, expressions of defense-related genes *PR-1*, *PR-5*, and *PDF1.2* were upregulated in the *ATAF1*-SRDX plants but attenuated or unchanged in the *ATAF1*-OE plants. In *ATAF1*-OE plants, SA-induced expression of pathogenesis-related genes and disease resistance against *P. syringae* pv. *tomato* DC3000 was partially suppressed. Increased levels of reactive oxygen species (i.e., H₂O₂ and superoxide anion) accumulated only in the *ATAF1*-OE but not in the *ATAF1*-SRDX plants after *Botrytis* spp. infection. Our studies provide direct genetic evidence for the role of ATAF1 as a negative regulator of defense response against different type of pathogens.

During coevolution with potential microbial pathogens, plants have developed a complex and precisely regulated network of genes to defend themselves against potential invading pathogens. A battery of defense mechanisms and defense genes are coordinately expressed in response to infection. Extensive biochemical, genetic, and genomic studies in *Arabidopsis* have revealed that salicylic acid (SA)- and jasmonic acid/ethylene (JA/ET)-dependent signaling pathways mediate the expression of defense genes and disease resistance responses (Glazebrook 2005). Generally, the speed and intensity of activation of defense gene expression upon pathogen recognition is critical to establish effective defense responses. In recent years, increas-

ing evidence has implicated a range of transcription factors directly or indirectly regulating plant defense responses against pathogens. Transcription factors are grouped into different families on the basis of conserved structural domains that are involved in DNA binding activity. Dozens of transcription factors belonging to the WRKY, ERF, MYB, and bZIP families play important roles in regulation of defense responses (Eulgem and Somssich 2007; Gutterson and Reuber 2004).

NAM, ATAF, and CUC (NAC) transcription factors comprise a large family of proteins, which contain a highly conserved N-terminal DNA-binding domain and a variable C-terminal domain (Olsen et al. 2005). Bioinformatics analyses have identified more than 100 NAC genes existing in the genomes of *Arabidopsis* and rice. (Ooka et al. 2003; Fang et al. 2008). NAC proteins appear to be unique transcription factors in plants and no homolog has been identified in other eukaryotes thus far (Olsen et al. 2005). Recent studies have implicated NAC proteins as important components in different aspects of plant development, including formation of boundary cells of the meristem, cell division and expansion, lateral root development, senescence, secondary cell wall biosynthesis, and flowering time (Souer et al. 1996; Aida et al. 1997; Sablowski and Meyerowitz 1998; Xie et al. 2000; Guo and Gan 2006; Kim et al. 2006; Zhong et al. 2006; Mitsuda et al. 2005, 2007; Yoo et al. 2007). On the other hand, several NAC proteins from *Arabidopsis* and rice are believed to be important in the control of responses to abiotic stresses. At least four *Arabidopsis* dehydration-inducible NAC genes—ANAC019, ANAC055, ANAC072 (RD26), and ATAF1—and three rice drought stress-inducible NAC genes—SNAC1, SNAC2, and OsNAC6—are associated with drought tolerance, and overexpression of some of these genes in plants confers enhanced drought tolerance (Tran et al. 2004, 2007; Fujita et al. 2004; Hu et al. 2006, 2008; Lu et al. 2007; Nakashima et al. 2008; Zheng et al. 2009). *Arabidopsis* AtNAC2/ANAC092 and NTL8/NTL9 are involved in salt and osmotic stress responses (He et al. 2005; Kim et al. 2008; Yoon et al. 2008). Moreover, NAC proteins are also involved in iron homeostasis in plants (Uauy et al. 2006; Ogo et al. 2008).

Accumulating evidence indicates that the NAC family proteins play important roles in regulation of plant defense response against pathogens. The first line of evidence came from the potato gene *StNAC*, which was induced by pathogen attack (Collinge and Boller 2001). It was found that some NAC genes from rapeseed, rice, barley, pepper, and *Arabidopsis* are induced by pathogen infection or treatment with defense signal molecules (Hegedus et al. 2003; Delessert et al. 2005; Oh et al.

Corresponding author: Fengming Song; E-mail: fmsong@zju.edu.cn; Fax: +086-0571-86049815.

2005; Ohnishi et al. 2005; Jensen et al. 2007). Functional analyses using T-DNA insertion lines and overexpression in transgenic plants revealed that *Arabidopsis* ATAF2 and ANAC019/ANAC055 genes are negative regulators of defense response against the vascular wilt pathogen, *Fusarium oxysporum*, and the necrotrophic pathogen, *Botrytis cinerea*, through suppression of defense gene expression (Delessert et al. 2005; Bu et al. 2008). It was also found that *Arabidopsis* ATAF1 and its barley homologue HvNAC6 contribute to penetration resistance in *Arabidopsis* against the nonhost pathogen, *Blumeria graminis* f. sp. *hordei*, and in barley against virulent *B. graminis* f. sp. *hordei* (Jensen et al. 2007, 2008). Furthermore, some NAC proteins are also involved in plant-virus interactions by interacting with viral components, and disruption of such NAC proteins in rice confers enhanced disease resistance against *Rice dwarf virus* (Ren et al. 2000; Selth et al. 2005; Jeong et al. 2008; Yoshii et al. 2008). Thus, the NAC proteins have diverse functions in plant disease resistance.

Recently, it was found that ATAF1, one of the first identified NAC transcription factors in *Arabidopsis*, negatively regulates drought tolerance but plays an important role in penetration resistance against the nonhost pathogen *B. graminis* f. sp. *hordei* in *Arabidopsis* (Jensen et al. 2007, 2008; Lu et al. 2007). In the present study, we studied the function of *Arabidopsis*

ATAF1 in regulation of defense responses. The disease phenotype of T-DNA insertion mutant, overexpression, and chimeric repressor transgenic plants were analyzed. Expression of *ATAF1* was downregulated after infection with *Botrytis cinerea* or *Pseudomonas syringae* pv. *tomato* DC3000 or after treatment with SA and JA. Transgenic plants with overexpression of the *ATAF1* gene (*ATAF1*-OE) showed increased susceptibility while transgenic plants expressing an *ATAF1* chimeric repressor construct (*ATAF1*-SRDX) exhibited enhanced resistance against *P. syringae* pv. *tomato* DC3000, *B. cinerea*, and *Alternaria brassicicola*. After infections by *B. cinerea* or *P. syringae* pv. *tomato* DC3000, expression of defense genes was upregulated in the *ATAF1*-SRDX plants but downregulated in the *ATAF1*-OE plants. SA-induced expression of pathogenesis-related (*PR*) genes and disease resistance against *P. syringae* pv. *tomato* DC3000 was partially suppressed in *ATAF1*-OE plants. Reactive oxygen species (ROS) accumulated only in the *ATAF1*-OE but not in the *ATAF1*-SRDX plants after *Botrytis* spp. infection. Our studies provide direct genetic evidence for the role of ATAF1 as a negative regulator of defense against different type of pathogens.

RESULTS

Downregulation of *ATAF1* expression in response to pathogen infection and inducer treatment.

The *ATAF1* gene encodes a 289-amino-acid protein with a conserved NAC domain at its N terminus. Phylogenetic tree analysis revealed that ATAF1 has approximately 59.5 to 71.1% identity with ANAC032 (At1g77450), ATAF2 (ANAC081, At5g08790), and ANAC102 (At5g63790). Only ATAF2 and ATAF1 have been previously reported to be involved in defense against *Fusarium* wilt disease, penetration resistance against nonhost pathogen *Blumeria graminis* f. sp. *hordei*, and drought tolerance (Delessert et al. 2005; Lu et al. 2007; Jensen et al. 2007, 2008), whereas no biological function has been assigned to ANAC032 and ANAC102. This implies that this small subgroup of NAC proteins may function against biotic and abiotic stress responses.

To explore the possible involvement of *ATAF1* in disease resistance, we analyzed its expression patterns after infection by different pathogens, including *B. cinerea* and *P. syringae* pv. *tomato* DC3000, in wild-type (WT) and mutant plants with altered disease responses. Expression level of the *ATAF1* gene was reduced in WT plants after inoculation with *B. cinerea*, and this reduction was quite evident at 48 h after inoculation compared with the expression levels in mock-inoculated plants (Fig. 1A). The membrane-anchored *BOTRYTIS-INDUCED KINASE1* gene is required for disease resistance against *B. cinerea* and the *bik1* mutant shows severe susceptibility to necrotrophic fungal pathogens, including *B. cinerea* (Veronese et al. 2006). However, *ATAF1* expression was unchanged in the *bik1* plants after *Botrytis* spp. infection and the expression levels were comparable with the levels in mock-inoculated plants during a period of 48 h after inoculation (Fig. 1A). Similarly, expression levels of the *ATAF1* gene were decreased in WT plants 12 h after inoculation with *P. syringae* pv. *tomato* DC3000 and the expression was significantly reduced at 24 h after inoculation compared with those in mock-inoculated plants (Fig. 1B). However, expression of the *ATAF1* gene showed distinct kinetics in *NahG* and *npr1* plants after infection of *P. syringae* pv. *tomato* DC3000. The *nahG* plants fail to accumulate SA whereas *npr1* plants are altered in SA signaling; thus, these plant lines represent critical components of the SA-mediated defense response pathway. Compared with the mock-inoculated plants, the *NahG* plants showed only slight reduction in the expression level of *ATAF1* after inoculation;

Fig. 1. Expression of *ATAF1* gene is downregulated in *Arabidopsis* plants in response to pathogen infection and chemical treatments. **A** and **B**, Expression of *ATAF1* in wild-type (WT) and mutant plants in responses to **A**, *Botrytis cinerea* and **B**, *Pseudomonas syringae* pv. *tomato* DC3000 infection. Four-week-old WT (Col-0) and mutant (*bik1*, *NahG*, and *npr1*) plants were inoculated by spraying with spores of *B. cinerea* (2×10^5 spores/ml), by infiltration with *P. syringae* pv. *tomato* DC3000 (optical density at 600 nm = 0.002), and by treatment with corresponding solutions. The inoculated leaves were collected at indicated times after inoculation for RNA isolation. Expression of *ATAF1* was analyzed using reverse-transcriptase polymerase chain reaction (RT-PCR) (32 cycles for *ATAF1* and 26 cycles for *actin* gene) with gene-specific primers. **C** and **D**, Expression of *ATAF1* in WT and mutant plant responses to treatments with chemical inducers. Four-week-old WT (Col-0) and mutant (*NahG* and *npr1*) plants were treated by foliar spraying with 1 mM salicylic acid (SA), 0.3 mM benzothiadiazole (BTH), 100 μ M jasmonic acid (JA), 100 μ M ACC, or water as controls. Sample collection and RT-PCR analysis of *ATAF1* expression were performed as in **A** and **B**. Samples for 0-h controls were collected immediately after inoculation or treatment.

whereas the *npr1* mutant plants showed a significant reduction in expression of *ATAF1*, especially at 24 h after inoculation (Fig. 1B). These results indicate that expression of the *ATAF1* gene is downregulated in response to pathogen infection and its downregulation depends on SA accumulation and signaling.

We next analyzed the expression patterns of the *ATAF1* gene in response to treatments of defense-inducing chemicals. We first compared the expression kinetics of the *ATAF1* gene in WT, *NahG*, and *npr1* plants after SA treatment. Similar to the expression pattern in WT plants after *P. syringae* pv. *tomato* DC3000 infection, SA treatment suppressed *ATAF1* expression compared with the levels in water-treated control plants. The expression of the *ATAF1* gene was decreased significantly at 12 h after inoculation and a further decrease in expression was observed at 24 h after inoculation (Fig. 1C). In *npr1* plants, SA treatment also suppressed *ATAF1* expression and the expression pattern of the *ATAF1* gene was similar to that in WT plants after SA treatment (Fig. 1C). However, less reduction of *ATAF1* expression was observed in *NahG* plants after SA treatment (Fig. 1C). Treatment of WT plants with benzothiadiazole (BTH), an analog of SA, also resulted in a significant reduction of *ATAF1* expression (Fig. 1D). On the other hand, decreases in *ATAF1* expression were also observed in WT plants after treatment with JA and 1-aminocyclopropane-1-carboxylate (ACC), although decreases of *ATAF1* expression were much more evident in JA-treated plants than that in ACC-treated ones. Together, these results indicate that expression of the *ATAF1* gene is also downregulated in response to molecules that induce the SA and JA/ET signaling pathways.

Identification of *ataf1*, ATAF1-SRDX, and ATAF1-OE lines.

To determine the biological role of the *ATAF1* gene directly, we identified and characterized a T-DNA knockout mutant for the *ATAF1* gene. SALK_057618 contains a T-DNA insertion in the third exon of the *ATAF1* gene and was designated as *ataf1-2* (Fig. 2A), consistent with the previous reports (Lu et al. 2007; Jensen et al. 2007). Homozygous mutant plants were identified by genotyping with *ATAF1*-specific primers flanking the insertion sites (Fig. 2B). Reverse-transcriptase polymerase chain reaction (RT-PCR) analysis revealed that no full-length *ATAF1* transcripts were detected in *ataf1-2* homozygous plants and that *ATAF1* was expressed normally in heterozygous and WT plants (Fig. 2C). This result indicated that *ataf1-2* is a null mutant of the *ATAF1* gene. The *ataf1-2* plants exhibited no detectable alteration in morphology (Fig. 2F). However, when tested for disease resistance against *B. cinerea* and *P. syringae* pv. *tomato* DC3000, no significant difference was observed in *ataf1-2* plants compared with WT plants (see below).

Chimeric repressor silencing technology was used to overcome functional redundancy of genes that may compensate for the *ATAF1* gene in the *ataf1* mutant (Hiratsu et al. 2003). To do this, the coding sequence of the *ATAF1* gene was artificially fused to a plant-specific transcriptional repression domain (LDLDLELRGFA), named "SRDX", at its C terminus, and this construct was cloned into a binary vector under the control of the *Cauliflower mosaic virus* (CaMV) 35S promoter, resulting in an ATAF1-SRDX construct (Fig. 2D). In addition, a CaMV 35S promoter-driven overexpression construct was also made by inserting the *ATAF1* coding sequence into a plant binary vector to make an ATAF1-OE construct. The ATAF1-SRDX and ATAF1-OE constructs were introduced into WT (Col-0) *Arabidopsis* plants through floral dip transformation and transgenic lines that constitutively express ATAF1-SRDX or ATAF1 itself were obtained. Homozygous T3 lines with a single locus of the transgene were screened from T2 seeds based on a 3:1 (Hgr-resistant/Hgr-sensitive) segregation ratio

on selective medium. Two lines each for ATAF1-SRDX (ATAF1-SRDX nos. 3 and 10) and ATAF1-OE (ATAF1-OE nos. 1 and 7) were chosen for further studies. RT-PCR analysis using *ATAF1*-specific primers revealed that the expression levels of the *ATAF1* gene were higher in the ATAF1-SRDX and ATAF1-OE plants than those in WT and vector-transformed plants (Fig. 2E), indicating that the transgene is expressed correctly. When RT-PCR was performed using an *ATAF1*-specific primer and a STD primer, only expression of the ATAF1-SRDX transcript was detected in ATAF1-SRDX plants (Fig. 2E). During our studies, no significant morphological or growth or development changes were observed in the ATAF1-SRDX and ATAF1-OE transgenic plants (Fig. 2F).

Altered disease phenotypes of ATAF1-OE and ATAF1-SRDX transgenic plants against *B. cinerea* and *A. brassicicola*.

To explore the possible role of ATAF1 in disease resistance response, we first compared the phenotype of the ATAF1-OE,

Fig. 2. Characterization of *ataf1-2*, ATAF1-SRDX, and ATAF1-OE lines. **A**, through **C**, Characterization of the *ataf1-2* T-DNA insertion mutant. **A**, The T-DNA insertion is located in the third exon of the *ATAF1* gene. **B**, Genotyping for homozygous was performed using gene-specific primers and a T-DNA primer. **C**, Expression level of the *ATAF1* gene in *ataf1-2* mutant under normal growth condition was analyzed using reverse-transcriptase polymerase chain reaction (RT-PCR) with gene-specific primers. Ht, heterozygous; Ho, homozygous; WT, wild-type. **D** through **E**, Characterization of ATAF1-SRDX and ATAF1-OE lines. **D**, Diagram of the construct used for expression of the chimeric ATAF1 repressor was presented. SRDX, repression domain; CaMV 35S, 35S promoter of *Cauliflower mosaic virus*; Nos, nopaline synthase terminator. **E**, Levels of the *ATAF1* and *ATAF1-SRDX* transcripts were analyzed using RT-PCR with a pair of gene-specific primers for *ATAF1* transcript and a pair of a gene-specific primer and a SRDX primer for *ATAF1-SRDX* transcript, respectively. **F**, Morphology of representative 4-week-old WT, *ataf1-2* mutant, ATAF1-SRDX, and ATAF1-OE plants.

ataf1-2, and ATAF1-SRDX plants against two necrotrophic fungal pathogens, *B. cinerea* and *A. brassicicola*. In *B. cinerea* assays, 4-week-old plants were inoculated by spraying a suspension containing 2×10^5 spores/ml, as described previously (Mengiste et al. 2003; Veronese et al. 2006). In WT plants, typical disease symptoms (i.e., necrotic and chlorotic lesions) were observed 5 days after inoculation (Fig. 3A). However, the necrotic and chlorotic lesions were restricted without leaf damage during the 10-day experimental period and relative chlorophyll contents in leaves of the inoculated plants were reduced by approximately 25% at 10 days after inoculation (Fig. 3A and B). Approximately 10% of the inoculated WT plants were completely decayed due to *B. cinerea* infection at 12 days after inoculation (Fig. 3C). In contrast, the ATAF1-OE

plants exhibited severe susceptibility to *B. cinerea*. At 5 days after inoculation, large necrotic and chlorotic lesions that resulted in extensive tissue damage appeared on leaves of the ATAF1-OE no. 3 and 10 plants, resulting in extensive tissue damage (Fig. 3A). The relative chlorophyll contents in leaves of the ATAF1-OE no. 1 and 7 plants were significantly decreased, leading to a reduction of approximately 45 to 50% (Fig. 3B), and approximately 55 to 65% of the inoculated plants were completely decayed at 12 days after inoculation (Fig. 3C). However, the ATAF1-SRDX plants showed fewer disease symptoms. No significant necrotic and chlorotic lesions were observed on leaves of the ATAF1-SRDX no. 3 and 10 plants even at 10 days after inoculation (Fig. 3A). The relative chlorophyll content in leaves of the ATAF1-SRDX no. 3 and 10 plants was slightly reduced (approximately 15%) and only approximately 7% of the inoculated plants were completely decayed at 12 days after inoculation (Fig. 3B and C). Disease levels in the *ataf1-2* plants were slightly higher than those in the ATAF1-SRDX plants but were somewhat less than WT plants (Fig. 3A). The relative chlorophyll contents in leaves of the *ataf1-2* plants were reduced by approximately 20% and only approximately 7% of the inoculated plants were completely decayed at 12 days after inoculation (Fig. 3B and C).

In planta growth of *B. cinerea* in inoculated plants was assessed to further confirm the phenotype observed in the disease assays. The accumulation of the *B. cinerea* actin A (*BcActin-A*) gene transcript was determined and compared with WT, ATAF1-OE, ATAF1-SRDX, and *ataf1-2* plants using RT-PCR. The *BcActin-A* gene was shown previously to be expressed constitutively in *B. cinerea* during plant infection (Benito et al. 1998); therefore, the amount of its transcript is indicative of the rate of fungal growth in planta. Accumulation of the *BcActin-A* gene transcript in WT, ATAF1-OE, ATAF1-SRDX, and *ataf1-2* plants were correlated well with the disease symptoms (Fig. 3D). In WT plants, the *BcActin-A* transcript was detected at 12 h after inoculation and increased in abundance as infection advanced (Fig. 3D). In the ATAF1-OE plants, the *BcActin-A* transcript increased faster than in WT plants within 24 h after inoculation and accumulated to significantly higher levels at 2 days after inoculation (Fig. 3D). However, significantly less of the *BcActin-A* gene transcript accumulated at 12 h after inoculation in the *ataf1-2* and ATAF1-SRDX plants. At 2 days after inoculation, the *BcActin-A* gene transcript in the *ataf1-2* plants was higher than that in the ATAF1-SRDX plants but was less than WT plants (Fig. 3D). Taken together, these data indicate that overexpression of ATAF1 resulted in increased susceptibility to *B. cinerea* and disruption of ATAF1 led to enhanced resistance to *B. cinerea*, as determined by both the disease phenotype and fungal growth in plant tissue.

We further tested whether altered ATAF1 function affected disease phenotype against another necrotrophic fungal pathogen, *A. brassicicola*, the causal agent of early blight in cruciferous plants. In these experiments, detached leaves from 4-week-old WT, ATAF1-OE, *ataf1-2*, and ATAF1-SRDX plants were drop inoculated with 5 μ l of spore suspension (5×10^5 spores/ml). In leaves of WT plants, necrotic lesions spread only slightly beyond the point of inoculation and were surrounded by yellow chlorotic zones (Fig. 4A). The lesion sizes measured approximately 4.5 mm in diameter at 6 days after inoculation (Fig. 4B) and the fungus produced approximately 5,100 spores per lesion at 5 days after inoculation (Fig. 4C). A significant increase in disease symptoms was observed in leaves of the ATAF1-OE plants. The necrotic lesions, surrounded by extensive chlorotic yellowish zones, spread markedly beyond the inoculation sites and the lesion sizes were approximately 6.2 to 6.7 mm in diameter in the ATAF1-OE no. 1

Fig. 3. Altered disease responses of *ataf1-2*, ATAF1-SRDX, and ATAF1-OE plants to *Botrytis cinerea*. **A**, Representatives of disease symptom on *ataf1-2*, ATAF1-SRDX, and ATAF1-OE plants. Four-week-old plants were inoculated by spraying with spores of *B. cinerea* (2×10^5 spores/ml) and photos were taken 5 days after inoculation. **B**, Changes in chlorophyll content in leaves of wild-type (WT), *ataf1-2*, ATAF1-SRDX, and ATAF1-OE plants after *B. cinerea* infection. Chlorophyll content in inoculated plants is expressed as percentages of the chlorophyll in the corresponding mock-inoculated plants. Chlorophyll was extracted from entire rosette leaves of six mock-inoculated and six *B. cinerea*-inoculated plants for each line. Data are the means from three independent experiments. **C**, Percent decay of WT, *ataf1-2*, ATAF1-SRDX, and ATAF1-OE plants after *B. cinerea* infection. Plants were scored as decayed when they were completely rotten. Data represented are the means from three different experiments with at least 30 plants per line for each experiment. **D**, In planta growth of *B. cinerea* in WT, *ataf1-2*, ATAF1-SRDX, and ATAF1-OE plants. Fungal growth was measured by analyzing expression level of *B. cinerea* actin A (*BcActinA*) gene using reverse-transcriptase polymerase chain reaction (RT-PCR) with *Arabidopsis* actin mRNA as a control. RT-PCR was performed using the *Botrytis* and *Arabidopsis* actin gene-specific primers, respectively. The experiments were repeated three times, with similar results.

and 7 plants (Fig. 4A and B). The fungus produced approximately 14,100 to 15,200 spores per lesion in the ATAF1-OE no. 1 and 7 plants at 5 days after inoculation, resulting in an increase of approximately 176 to 198% compared with that in WT plants (Fig. 4C). However, in the ATAF1-SRDX no. 3 and 10 plants, only small necrotic lesions appeared at the inoculation sites and these were not surrounded by chlorotic yellowish zone (Fig. 4A). The sizes of the necrotic lesions measured approximately 2.0 to 2.1 mm in diameter, resulting in a reduction of approximately 55%, and the fungus produced approximately 3,100 to 3,700 spores per lesion, resulting in a reduction of approximately 27 to 40% compared with those in WT plants, respectively (Fig. 4B and C). Necrotic lesions surrounded by slight chlorotic yellowish zones in leaves of the *ataf1-2* plants were observed (Fig. 4A). The lesion sizes were approximately 2.9 mm in diameter and the fungus produced approximately 4,000 spores per lesion, slightly larger or more than those in the ATAF1-SRDX plants but slightly smaller or less than those in WT plants, respectively (Fig. 4B and C). These data confirm that overexpression of *ATAF1* results in increased susceptibility to *A. brassicicola*, and that disruption of *ATAF1* led to enhanced resistance to *A. brassicicola*. The increased susceptibility of the ATAF1-OE plants and enhanced resistance of ATAF1-SRDX and *ataf1-2* plants to both *B. cinerea* and *A. brassicicola* suggest a broad role for ATAF1 in resistance to necrotrophic fungal pathogens.

Altered disease phenotypes of ATAF1-OE and ATAF1-SRDX transgenic plants against *P. syringae* pv. *tomato* DC3000.

We next studied the possible function of ATAF1 in resistance against a bacterial pathogen by analyzing disease phenotypes of the different ATAF1 lines to *P. syringae* pv. *tomato* DC3000 inoculation. Four-week-old plants were challenged with a normally virulent strain of *P. syringae* pv. *tomato*

DC3000 and disease phenotypes were compared. In WT plants, typical chlorotic lesions were observed and bacterial populations in inoculated leaves were 3.2×10^6 and 1.6×10^7 CFU/cm² at 2 and 4 days after inoculation, respectively (Fig. 5A and B). The ATAF1-OE plants developed more severe disease symptoms than WT plants after inoculation with the bacterial pathogen, resulting in extensive chlorotic lesions on the inoculated leaves (Fig. 5A). Similarly, bacterial populations in inoculated leaves of the ATAF1-OE no. 1 and 7 plants were approximately 7.8 to 10.0×10^7 and approximately 6.3 to 12.6×10^8 CFU/cm² at 2 and 4 days after inoculation, respectively, resulting in a significant increase (approximately 24- to 79-fold) in bacterial growth relative to WT plants (Fig. 5B). By contrast, only very small chlorotic lesions were observed in inoculated leaves of the ATAF1-SRDX plants, and the bacterial populations in inoculated leaves of the ATAF1-SRDX no. 3 and 10 plants were approximately 6.4 to 8.0×10^5 and approximately 1.6 to 2.5×10^6 CFU/cm² at 2 and 4 days after inoculation, respectively, resulting in a significant decrease (approximately four- to sixfold) in bacterial growth compared with WT plants (Fig. 5A and B). Small necrotic lesions appeared in leaves of the *ataf1-2* plants and the bacterial populations in inoculated leaves of the *ataf1-2* plants were 1.0×10^6 and 5.0×10^6 CFU/cm² at 2 and 4 days after inoculation, respectively (Fig. 5A and B), leading to an approximately threefold decrease in bacterial population compared with WT plants. Disease levels in the *ataf1-2* plants were higher than those in the ATAF1-SRDX plants but were slightly lower than those in WT plants. These results indicate that constitutive

Fig. 4. Altered disease responses of the *ataf1-2*, ATAF1-SRDX, and ATAF1-OE plants to *Alternaria brassicicola*. **A**, Representative disease symptom in *ataf1-2*, ATAF1-SRDX, and ATAF1-OE plants. Detached leaves from 4-week-old plants were inoculated with a 5- μ l droplet of *A. brassicicola* spores (1×10^6 spores/ml) and photographed 5 days after inoculation. **B**, Lesion size on leaves of WT, *ataf1-2*, ATAF1-SRDX, and ATAF1-OE plants. Lesion size was measured at 5 days after inoculation and data presented are the means $\pm$ standard deviation from at least 40 lesions. **C**, Sporulation of *A. brassicicola* on leaves of the WT, *ataf1-2*, ATAF1-SRDX, and ATAF1-OE plants. Spores were collected from detached inoculated leaves and counted microscopically at 6 days after inoculation. Data represented are the means $\pm$ standard deviation from three independent experiments with at least 20 inoculated leaves taken from each experiment.

Fig. 5. Altered disease responses in *ataf1-2*, ATAF1-SRDX, and ATAF1-OE plants to *Pseudomonas syringae* pv. *tomato* DC3000. **A**, Representative disease symptom on *ataf1-2*, ATAF1-SRDX, and ATAF1-OE plants. Four-week-old plants were inoculated with infiltration with *P. syringae* pv. *tomato* DC3000 (optical density at 600 nm = 0.002). Photos were taken 4 days after inoculation. **B**, Bacterial growth in inoculated leaves of WT, *ataf1-2*, ATAF1-SRDX, and ATAF1-OE plants. Leaf samples were collected at 0, 2, and 4 days after inoculation and bacterial growth in CFU/cm² leaf area was measured. Data presented are the means $\pm$ standard deviation from three independent experiments.

overexpression of *ATAF1* resulted in increased growth of the bacteria and enhanced development of disease symptoms and that disruption of *ATAF1* led to decreased growth of the bacteria and reduced development of disease symptoms. Therefore, *ATAF1* plays a role in resistance against *P. syringae* pv. *tomato* DC3000 in *Arabidopsis*.

Different patterns of defense gene expression in the *ATAF1*-OE and *ATAF1*-SRDX plants after pathogen infection.

To determine whether the altered disease responses of different types of pathogens are linked to changes in the regulation of defense genes, we studied the expression of some defense genes (i.e., *PR-1*, *PR-3*, *PR-5*, and *PDF1.2*) in WT, *ATAF1*-OE, and *ATAF1*-SRDX plants grown under normal conditions. No significant difference in expression of the defense genes tested was detected in WT, *ATAF1*-OE, and *ATAF1*-SRDX plants grown under normal conditions (data not shown). These results indicate that an altered function of *ATAF1* itself did not result in any defense response changes in plants, which is consistent with the notion that both the *ATAF1*-OE and *ATAF1*-SRDX plants have no visible morphological and growth or development changes.

We next analyzed and compared the expression levels of defense-related genes in response to *P. syringae* pv. *tomato* DC3000 infection. In WT plants, the expression levels of the *PR-1*, *PR-3*, *PR-5*, and *PDF1.2* genes as well as *NPR1* (a criti-

cal component of the SA signaling pathway) were induced after inoculation (Fig. 6A). When compared with those in WT plants, however, the expression levels of *PR-1*, *PR-5*, and *NPR1* genes were increased in the *ATAF1*-SRDX plants but reduced in the *ATAF1*-OE plants after inoculation (Fig. 6A). Expression of *PR-3* was unchanged in WT, *ATAF1*-SRDX, and *ATAF1*-OE plants after infection. When compared with those in WT plants, however, the expression levels of *PDF1.2* were reduced in the *ATAF1*-SRDX plants but increased in the *ATAF1*-OE plants after infection (Fig. 6A).

We further compared the expression levels of defense-related genes in response to *Botrytis* spp. inoculation. After inoculation with *B. cinerea*, expression of the *PR-1*, *PR-3*, *PR-5*, and *PDF1.2* genes as well as *BIK1* was induced in WT plants (Fig. 6B). However, the expression levels of *PR-1*, *PR-5*, and *PDF1.2* genes were increased in the *ATAF1*-SRDX plants but reduced in the *ATAF1*-OE plants compared with those in WT plants after infection (Fig. 6B). The expression level of *PR-3* was comparable among WT, *ATAF1*-SRDX, and *ATAF1*-OE plants after infection. After *Botrytis* spp. infection, the expression levels of *BIK1* in the *ATAF1*-SRDX plants were similar to those in WT plants, but the level in the *ATAF1*-OE plants was increased (Fig. 6B).

Collectively, these data indicate that enhanced resistance of the *ATAF1*-SRDX plants against *P. syringae* pv. *tomato* DC3000 and *B. cinerea* may be ascribed to the increased expression of defense genes after pathogen infection and that increased susceptibility of the *ATAF1*-OE plants may be a result of loss of activation of defense gene expression upon pathogen infection.

Attenuation of SA-induced defense response in *ATAF1*-OE plants.

Because expression of *ATAF1* was downregulated by SA and expression of *NPR1* in *ATAF1*-OE plants was upregulated after infection by *P. syringae* pv. *tomato* DC3000, we hypothesized that *ATAF1* might have a function in SA-induced disease resistance response. To test this hypothesis, we compared the expression of *PR-1* and *PR-5* genes in WT and *ATAF1*-OE plants after SA treatment. In WT plants, SA significantly induced expression of *PR-1* and *PR-5* genes at 24 h after treatment (Fig. 7A). Although SA also induced expression of *PR-1* and *PR-5* in *ATAF1*-OE no. 1 and 7 plants, their expression was lower than in WT plants. Furthermore, SA-induced disease resistance in the *ATAF1*-OE plants was also assessed by comparing disease phenotype and bacterial population in water- and SA-treated WT and *ATAF1*-OE plants after inoculation with *P. syringae* pv. *tomato* DC3000. For this purpose, water- and SA-treated WT and *ATAF1*-OE plants were inoculated by infiltration with *P. syringae* pv. *tomato* DC3000 at 24 h after SA treatment. The SA-treated WT plants showed less disease than the water-treated plants at 4 days after inoculation. Disease phenotypes in the SA-treated *ATAF1*-OE plants were comparable with those in water-treated plants but were much more severe than those in WT plants (Fig. 7B). At 3 days after inoculation, the bacterial populations were measured as 1.21×10^7 CFU/cm² and approximately 1.12 to 1.16×10^8 CFU/cm² in water-treated WT and *ATAF1*-OE plants, and as 1.15×10^6 CFU/cm² and approximately 1.58 to 1.76×10^7 CFU/cm² in SA-treated WT and *ATAF1*-OE plants, respectively (Fig. 7C). The ratio of bacterial populations in water-treated/SA-treated WT plants was 10.52, whereas this ratio was approximately 6.59 to 7.08 for the *ATAF1*-OE plants, indicating a relatively higher level of bacterial population in the *ATAF1*-OE plants than in the WT plants. These results suggest that the SA-induced disease resistance was attenuated in *ATAF1*-OE plants upon SA treatment.

Fig. 6. Expression of defense genes in wild-type (WT), *ataf1-2*, *ATAF1*-SRDX, and *ATAF1*-OE plants after inoculation with *Botrytis cinerea* and *Pseudomonas syringae* pv. *tomato* DC3000. Four-week-old plants were inoculated by **A**, infiltration with *P. syringae* pv. *tomato* DC3000 (optical density at 600 nm = 0.002) or **B**, spraying with spores of *B. cinerea*. Leaf samples were collected at indicated times after inoculation for RNA isolation. Expression of defense genes was analyzed using reverse-transcriptase polymerase chain reaction with gene-specific primers. Actin was used as a control. Two independent experiments were performed with similar results.

Accumulation of ROS in ATAF1-OE and ATAF1-SRDX plants after *Botrytis* spp. infection.

ROS have been implicated in susceptible responses against necrotrophic fungus (Mengiste et al. 2003; Veronese et al. 2006). We determined and compared the production of ROS in WT, ATAF1-OE, *ataf1-2*, and ATAF1-SRDX plants after *B. cinerea* inoculation. In situ H_2O_2 production was detected using

Fig. 7. Attenuation of salicylic acid (SA)-induced defense response in ATAF1-OE plants. **A**, Expression of pathogenesis-related (*PR*) genes in ATAF1-OE plants after SA treatment. Four-week-old wild-type (WT) and ATAF1-OE plants were treated by foliar spray of 1 mM SA and leaf samples were collected for reverse-transcriptase polymerase chain reaction analysis of *PR* gene expression. **B** and **C**, Partial suppression of SA-induced disease resistance against *P. syringae* pv. *tomato* DC3000 in ATAF1-OE plants. Four-week-old plants were treated by foliar spraying with 1 mM SA or water as control and infiltration-inoculated with *P. syringae* pv. *tomato* DC3000 (optical density at 600 nm = 0.002) 24 h later. **B**, Photos were taken 4 days after inoculation and **C**, bacterial titers were measured. Data presented are the means $\pm$ standard deviation from three independent experiments.

diaminobenzidine, which forms a visible reddish-brown polymer in tissue (Thordal-Christensen et al. 1997). The accumulation of superoxide anions (O_2^-) was tested by staining with nitroblue tetrazolium (Doke 1983). In mock-inoculated plants, no significant accumulation of H_2O_2 and O_2^- was observed (Fig. 8A and B), indicating that the plants used for the experiments were not stressed. At 48 h after inoculation with *B. cinerea*, significant accumulation of H_2O_2 and O_2^- was detected in leaves of WT plants (Fig. 8A and B). However, much more H_2O_2 and O_2^- were generated in leaves of the ATAF1-OE plants than in those of WT plants. By contrast, less H_2O_2 and O_2^- was detected in leaves of ATAF1-SRDX and *ataf1-2* plants,

Fig. 8. Comparison of reactive oxygen species accumulation in wild-type (WT), *ataf1-2*, ATAF1-SRDX, and ATAF1-OE plants after *Botrytis cinerea* inoculation. Four-week-old plants were inoculated by spraying with spores of *B. cinerea* or mock inoculated and leaf samples were taken 48 h after inoculation. Accumulation of H_2O_2 and O_2^- in leaves was detected by 3,3'-diaminobenzidine and nitroblue tetrazolium staining, respectively. The experiments were repeated twice with similar results.

especially in the ATAF1-SRDX plants, in which only limited staining of H₂O₂ and O₂⁻ was observed (Fig. 8A and B).

DISCUSSION

It has been shown recently that *Arabidopsis* ATAF1 acts as a negative regulator of drought tolerance and mediates efficient penetration resistance against nonhost pathogens by attenuation of abscissic acid (ABA) signaling (Lu et al. 2007; Jensen et al. 2007, 2008). In the present study, through comparative analysis of disease phenotypes in transgenic overexpression lines and transgenic chimeric repressor lines, we provide direct genetic evidence supporting ATAF1 functions as a negative regulator of plant disease resistance against both different types of pathogens.

Expression of *ATAF1* and its rice, barley, and potato homologues (*OsNAC6*, *HvNAC6*, and *StNAC*, respectively) is induced in response to pathogen infection as well as hormones (ABA and methyl JA) and abiotic stress (dehydration and wounding) (Collinge and Boller 2001; Schenk et al. 2003; Ohnishi et al. 2005; de Torres-Zabala et al. 2007; Jensen et al. 2007; Lu et al. 2007; Nakashima et al. 2007). Expression of *ATAF1* was only induced at 12 h during a 48-h period after infection with the nonhost pathogen *Blumeria graminis* f. sp. *hordei* and during late stage (after 48 h) of infection by *A. brassicicola* (Jensen et al. 2007; Schenk et al. 2003). In our study, however, we found that expression of *ATAF1* was downregulated in response to infection by *Botrytis cinerea* and *P. syringae* pv. *tomato* DC3000 as well as to treatments with SA and JA. This is similar to a previous observation for the transcription factor gene *AtWRKY48*, whose expression was rapidly suppressed by infection with *P. syringae* pv. *tomato* DC3000 (Xing et al. 2008). Interestingly, pathogen- or SA-induced downregulation of *ATAF1* expression showed different patterns in some mutant plants; for example, the expression level of *ATAF1* in *bik1* and *NahG* plants was not significantly downregulated after infection with *B. cinerea* or *P. syringae* pv. *tomato* DC3000 and SA treatment. Theoretically, a large set of genes encoding both positive and negative defense regulators is often coordinately activated in plants upon pathogen infection, and expression of those genes for negative regulators must be suppressed to activate efficient defense responses on time. Suppression of *ATAF1* expression may be one of the defense responses activated in plants after pathogen infection.

Functional redundancy in the NAC family is a very common phenomenon; for examples, *CUC1/CUC2* (Aida et al. 1997), *NST1/NST2* (Mitsuda et al. 2005), *ANAC019/ANAC055* (Bu et al. 2008), and *ATAF2/At5g63790* (Delessert et al. 2005) are functionally redundant pairs of NAC genes in *Arabidopsis*. T-DNA insertion mutants for individual members of the above-mentioned NAC pairs showed no visible phenotypic changes compared with WT plants, and altered phenotypes were observed only in double mutants of *cuc1/cuc2*, *nst1/nst2*, and *anac019/anac055* (Aida et al. 1997; Mitsuda et al. 2005; Bu et al. 2008). In our experiments, we were unable to observe significant changes in disease phenotypes in *ataf1-2* plants after inoculation with *B. cinerea* and *P. syringae* pv. *tomato* DC3000. This is similar to the observation for *ATAF2*, whose T-DNA-tagged knockout mutants did not show any change in disease resistance against *F. oxysporum* (Delessert et al. 2005). The chimeric repressor gene-silencing strategy can overcome functional redundancy of highly related genes and has been widely used in elucidating biological functions of a number of genes encoding transcriptional factors, including NAC proteins (Fujita et al. 2004; Mitsuda et al. 2005, 2007, 2008). In our study, ATAF1-SRDX plants showed increased disease resistance against infection with different pathogens than the *ataf1-*

2 plants, indicating that functional redundancy is also the case for the *ATAF1* gene. Phylogenetically, the ATAF1 protein has a high level of identity to At1g77450, whose function is not yet determined; thus, it is possible that disruption of *ATAF1* in *ataf1-2* plants may be compensated by At1g77450. With respect to this, it would be interesting to characterize the biological function of At1g77450 in biotic and abiotic stresses using overexpression and chimeric repressor transgenic plants as well as double mutants between the *ataf1* and *at1g77450* lines.

ATAF1 belongs to a small subfamily of NAC proteins called the ATAF subfamily. An *ATAF1* knockout mutant, *ataf1-1*, shows decreased penetration resistance to nonhost pathogen *Blumeria graminis* f. sp. *hordei* and ATAF1 acts as a new stimuli-dependent attenuator of ABA signaling for the mediation of efficient penetration resistance upon *B. graminis* f. sp. *hordei* infection (Jensen et al. 2007, 2008). In contrast to these observations, we found that disruption of ATAF1 function by chimeric dominant repression at protein activity levels conferred enhanced disease resistance against different types of adapted pathogens, whereas overexpression of *ATAF1* resulted in increased susceptibility. Our gain-of-function and loss-of-function evidence supports the idea that ATAF1 acts as a negative regulator of defense response. It has also been found that ATAF2, a member of the ATAF subfamily very closely related to ATAF1, and ANAC019/ANAC055 are negative regulators of defense response against *F. oxysporum* and *Botrytis cinerea*, respectively (Delessert et al. 2005; Bu et al. 2008). These observations imply that plants have developed distinct mechanisms to regulate basal resistance toward nonhost pathogens and defense responses against different types of adapted pathogens. This is supported by previous observations for the *Arabidopsis* *pen1* and *pen3* mutants, which are more susceptible to entry of the nonhost fungus *Blumeria graminis* f. sp. *hordei* but are more resistant to the *Arabidopsis* powdery mildew fungus *Erysiphe cichoracearum* (Stein et al. 2006; Zhang et al. 2007). Moreover, the ATAF1 homologues of the monocot plants (i.e., rice *OsNAC6* and barley *HvNAC6*) have been shown to play positive roles in regulation of defense responses. Silencing of *HvNAC6* compromises penetration resistance in barley epidermal cells toward virulent *B. graminis* f. sp. *hordei*, while transient overexpression of *HvNAC6* increases the occurrence of penetration-resistant cells toward *B. graminis* f. sp. *hordei* attack (Jensen et al. 2007). Similarly, overexpression of *OsNAC6* in rice resulted in slightly increased resistance to blast disease (Nakashima et al. 2007). Therefore, function of ATAF1 in disease resistance between dicot and monocot plants might also be different.

Defense responses against *P. syringae* pv. *tomato* DC3000 and necrotrophic pathogens are thought to be mediated by SA- and JA/ET-dependent signaling pathways, respectively (Glazebrook 2005). Both negative and positive cross-talk between JA/ET and SA signaling exist in plants in response to pathogen infections (Glazebrook 2005; Mur et al. 2006; Schenk et al. 2000). ATAF1 may have functions in both the SA- and JA/ET-dependent signaling pathways. This is supported by several lines of evidence obtained from our studies. First, expression of *ATAF1* was downregulated after infection by both *Botrytis cinerea* and *P. syringae* pv. *tomato* DC3000 and by treatments of SA and JA. Second, disruption of *ATAF1* function resulted in enhanced resistance against *P. syringae* pv. *tomato* DC3000 and necrotrophic pathogens, including *B. cinerea* and *A. brassicicola*, whereas overexpression of *ATAF1* led to increased susceptibility to these pathogens. Third, both SA- and JA-responsive defense genes were activated in ATAF1-SRDX plants upon infection with *P. syringae* pv. *tomato* DC3000 and *B. cinerea*. The function of ATAF1 in SA-mediated defense response is further confirmed by the observation that the SA-

and *P. syringae* pv. *tomato* DC3000-induced expression of SA-responsive *PR* genes and SA-induced disease resistance against *P. syringae* pv. *tomato* DC3000 was attenuated in the ATAF1-OE plants. The fact that expression of *ATAF1* in *NahG* plants was not downregulated as much as in WT and *npr1* plants after *P. syringae* pv. *tomato* DC3000 infection or SA treatment and expression of *NPR1* in the ATAF1-SRDX plants after infection of *P. syringae* pv. *tomato* DC3000 was further induced may suggest that ATAF1 has a function upstream of NPR1 but downstream of SA in the SA signaling pathway. However, genetic evidence is required to validate this possibility.

It was shown that BIK1 is required for activation of defense response against necrotrophic fungi, including *B. cinerea* and *A. brassicicola* (Veronese et al. 2005). Altered disease resistance, resulting from SRDX-mediated disruption of ATAF1 function and overexpression of *ATAF1* in WT plants, in which BIK1 has normal function as a positive regulator of *Botrytis* spp. resistance, provide no direct evidence regarding the genetic relationship between BIK1 and ATAF1 in modulating defense response against *B. cinerea*. The observations that expression of *ATAF1* was not suppressed in *bik1* plants after *Botrytis* spp. infection and that the ATAF1-OE plants showed increased susceptibility to *Botrytis* spp. suggest that ATAF1 may act downstream of BIK1. However, this is contradictory to some extent to the observation that expression of BIK1 was not induced by *B. cinerea* in ATAF1-SRDX plants. Therefore, it seems that a feedback interaction between function of ATAF1 and BIK1 is involved in regulation of defense response against *B. cinerea*. This will be clarified by further analysis of disease resistance phenotypes in transgenic plants constitutively expressing chimeric repressor construct ATAF1-SRDX in *bik1* background.

Neither growth or development defects nor constitutively activated defense response (expression of *PR* genes and accumulation of ROS) was observed in the ATAF1-SRDX plants, which showed enhanced disease resistance after pathogen infection. However, defense responses, including expression of *PR* genes, were activated in ATAF1-SRDX plants when challenged with *B. cinerea* and *P. syringae* pv. *tomato* DC3000. Production of ROS, which is related to susceptibility to necrotrophic pathogens, including *B. cinerea*, in some *Arabidopsis* mutants (Mengiste et al. 2003; Veronese et al. 2006), was suppressed in ATAF1-SRDX plants after inoculation with *B. cinerea*. It is likely that disruption of ATAF1 potentiates the rapid and strong activation of defense in response to attempted infection. This is similar to the observation for AtWRKY18, a pathogen- and SA-induced WRKY transcription factor, which potentiates developmentally regulated defense responses in transgenic plants when expressed at moderate levels (Chen and Chen 2000). On the other hand, expression of *PR* and *PDF1.2* genes was differentially upregulated in ATAF1-SRDX and ATAF1-OE plants upon infection with different pathogens. When inoculated with *P. syringae* pv. *tomato* DC3000, expression of some of the SA-responsive *PR* genes was upregulated, whereas expression of *PDF1.2* was suppressed in ATAF1-SRDX plants. In contrast, infection with *B. cinerea* induced significant expression of both SA-responsive and JA-responsive defense genes in ATAF1-SRDX plants, which is in agreement with previous observations that resistance to *B. cinerea* involves an SA signaling pathway and activation of *PR* gene expression in *Arabidopsis* (Govrin and Levine 2002; Ferrari et al. 2003; Zheng et al. 2006). Interestingly, a pathogen-specific effect on *PDF1.2* expression was observed, showing a positive correlation with *ATAF1* expression levels after *P. syringae* pv. *tomato* DC3000 infection and a negative correlation after *Botrytis* spp. infection. This might be ascribed to negative cross-

talk between SA- and ET/JA-signaling pathways that mediate the defense response against *P. syringae* pv. *tomato* DC3000 and necrotrophic pathogens, respectively (Glazebrook 2005). Thus, disruption of ATAF1 function in ATAF1-SRDX plants seems to potentiate a fine-tuned mechanism to efficiently regulate distinct defense responses upon infection with different pathogens.

Taken together, evidence from our study and others (Lu et al. 2007; Jensen et al. 2007, 2008) indicates that ATAF1 represents a node of convergence regulating plant defense and drought tolerance to multiple abiotic and biotic stress factors. Previous studies have shown that ATAF1 and its rice and barley homologues OsNAC6 and HvNAC6 are biochemically transcriptional activators rather than transcriptional repressors (Lu et al. 2007; Jensen et al. 2007; Nakashima et al. 2007). Therefore, it is unlikely that ATAF1 directly suppresses expression of downstream target genes. Instead, ATAF1 may first activate expression of some unknown negative regulators, which in turn repress expression of defense genes. Further identification of downstream target genes and their functions will provide new insights into how ATAF1 negatively regulates defense responses to different types of pathogens.

MATERIALS AND METHODS

Plant growth and treatment.

Arabidopsis thaliana WT ecotype Col-0, mutant, and transgenic plants were grown on soil in a growth room at 23°C under long-day conditions (cycle of 16 h of light and 8 h of darkness) with a relative humidity (RH) of 70%. For chemical treatments, SA (1 mmol/liter), BTH (300 µmol/liter), JA (100 µmol/liter), and ACC (100 µmol/liter) were prepared in water and applied as a spray to 4-week-old plants. The treated plants were kept at 100% RH and then harvested at the indicated times. Samples were collected at different time point and the 0-h samples were collected immediately after inoculation or treatment.

Plant inoculation and measurement of pathogen growth.

P. syringae pv. *tomato* DC3000 was grown overnight in King's B (KB) liquid medium containing rifampicin at 25 µg/ml, and the suspensions were diluted and regrown to an optical density at 600 nm (OD₆₀₀) of approximately 0.7 to 1.0. Bacteria were collected and resuspended in 10 mM MgCl₂ to OD₆₀₀ = 0.002 for plant inoculation. Four-week-old plants were infiltrated at two sites of two leaves with 20-µl aliquots of *P. syringae* pv. *tomato* DC3000 suspension or with MgCl₂ solution as mock inoculation using a 1-ml syringe without needle. After inoculation, plants were covered with a transparent plastic film and disease symptoms were observed daily. Six independent samples were used for the *P. syringae* growth curve measurement. Leaf punches (6 mm in diameter) were surface sterilized in 70% ethanol for 10 s, homogenized in 200 µl of 10 mM MgCl₂, diluted in 10 mM MgCl₂, and plated on KB agar plates containing rifampicin at 100 µg/ml.

B. cinerea was grown on 2× V8 agar (36% V8 juice, 0.2% CaCO₃, and 2% agar) at 20°C. Conidia were collected from 10-day-old cultures by placing agar slices containing fungal material in 1% maltose buffer and vortexing to release the spores. The spore suspension was passed through two layers of cheesecloth and spore density was adjusted to 2 × 10⁵ spores/ml. Inoculation was performed by foliar spraying with spore suspension in a fine mist onto leaves of 4-week-old plants. The inoculated plants were covered with a transparent plastic film and kept at 22°C in a growth chamber under a cycle of 14 h of light and 10 h of darkness. Chlorophyll contents were measured as described previously (Mengiste et al.

2003; Veronese et al. 2006) and expressed as percentages of the chlorophyll content in the corresponding mock-inoculated plants. Plants that were completely rotten were recorded as decayed plants. Fungal growth in leaf tissues was evaluated based on expression level of a *Botrytis* actin A (*BcActin* A) gene analyzed by RT-PCR using a pair of primers: *BcActinA*-1F (5'-ACT CAT ATG TTG GAG ATG AAG CGC AA-3') and *BcActinA*-1R (5'-AAT GTT ACC ATA CAA ATC CTT ACG GAC A-3'). Expression of an *Arabidopsis* actin gene (*AtActin*) as an internal control was also analyzed by RT-PCR with primers of *AtActin*-1F (5'-GGC GAT GAA GCT CAA TCC AAA CG-3') and *AtActin*-1R (5'-GGT CAC GAC CAG CAA GAT CAA GAC G-3').

A. brassicicola was grown on potato dextrose agar for 10 days at room temperature and spores were collected and resuspended in 0.1% Tween 20. Inoculation with *A. brassicicola* was performed by applying a 5- μ l drop of spore suspension (5×10^5 spores/ml) on each detached leaf of 4-week-old plants. Inoculated leaves were kept at 100% RH in sealed dishes. The diameter of each lesion was measured after 6 days. To count the number of spores, 10 leaves were collected and shaken vigorously in 5 ml of 0.1% Tween 20 in a test tube. Leaves were removed, and the spore-containing suspension was centrifuged at $5,000 \times g$ for 15 min. The spores were resuspended in 200 μ l of 0.1% Tween 20, serially diluted, and counted with a microscope.

Genotyping of the *ataf1* mutant.

A SALK T-DNA insertion line, SALK_057618, was obtained from the Arabidopsis Biological Resource Center at Ohio State University. Gene-specific primers were used for screening of homozygous were SALK_057618-LP (5'-TCC CAG GGA CAG AAA ATA TCC-3') and SALK_057618-RP (5'-AAA TAT TAA ATT GAT TGC GGC AC-3'). A T-DNA primer LBA1 (5'-TGG TTC ACG TAG TGG GCC ATC G-3') was also included, as suggested on the T-DNA Express Web site. A three-primer PCR strategy was used to identify WT, heterozygous, and homozygous individuals in a single step using two gene-specific primers and LBA1. Plants homozygous for the *ATAF1* mutation were used for further analysis.

Generation and characterization of the transgenic lines.

The cDNA of the *ATAF1* gene was amplified using RT-PCR with a pair of gene-specific primers, *ATAF1*-1F (5'-ATG TCA GAA TTA TTA CAG TTG CCT C-3') and *ATAF1*-1R (5'-CTA GTA AGG CTT CTG CAT GAT C-3'). To construct the over-expression plasmid, the coding region of *ATAF1* was amplified using a pair of primers, *ATAF1*-2F (5'-AGT GGA TCC ATG TCA GAA TTA TTA CAG TTG C-3') and *ATAF1*-2R (5'-GCG GGT ACC CTA GTA AGG CTT CTG CAT GTA C-3'), which contain a *Bam*HI site and a *Kpn*I site (underlined). To construct the *ATAF1* chimeric repressor plasmid, the coding region without the stop codon was amplified using a pair of primers, *ATAF1*-2F and *ATAF1*-3R (5'-GCG GGT ACC TTA GGC AAA TCC AAG GCG GAG CTC CAG ATC TAG GTC GAG GGG CCC GTA AGG CTT CTG CAT GTA CAT G-3'). The reverse primer contains an *Apa*I site and a synthetic SRDX (LDLDELRLGFA) coding sequence. The amplified fragments were cloned into the *Bam*HI/*Kpn*I or *Bam*HI/*Apa*I sites of binary vector pCambia 99-1 under control of the CaMV 35S promoter, respectively.

Arabidopsis transformation was performed using the floral dip method (Clough and Bent 1998). Seed from transformed plants (T0) were harvested and sown on half-strength Murashige-Skoog medium containing hygromycin (Hgr) at 50 μ g/ml. Transformants of the T1 generation were selected and self pollinated. The progenies of the T1 transformants were observed on selective medium and 3:1 (Hgr-resistant/Hgr-sen-

sitive) segregating lines were selected and transformed to soil for self pollination. Progeny (T2) of the individual T1 plants were observed on selective medium and those that showed 100% resistance to Hgr were selected as homozygous lines.

RT-PCR analysis of gene expression.

Total RNA from leaves was extracted using the TRIZOL reagent according to the manufacturer's procedure (Invitrogen, Shanghai). First-strand cDNA was synthesized from 500 ng of total RNA using superscript III reverse transcriptase (Invitrogen). Primers *ATAF1*-1F and -1R that amplify the full open reading frame fragment were used for detection of *ATAF1* expression. Other primers used in this study were *AtPR1*-F, 5'-TCG TCT TTG TAG CTC TTG TAG GTG-3'; *AtPR1*-R, 5'-TAG ATT CTC GTA ATC TCA GCT CT-3'; *AtPR5*-F, 5'-ATG GCA AAT ATC TCC AGT ATT CAC A-3'; *AtPR5*-R, 5'-ATG TCG GGG CAA GCC GCG TTG AGG-3'; *AtPDF1.2*-F, 5'-GCTA AGT TTG CTT CCA TCA TCA CCC TT-3'; and *AtPDF1.2*-R, 5'-AAC ATG GGA CGT AAC AGA TAC ACT TGT G-3'. Expression of a housekeeping gene (i.e., actin) was used as a control with a pair of primers, *AtActin*-1F (5'-GGC GAT GAA GCT CAA TCC AAA CG-3') and *AtActin*-1R (5'-GGT CAC GAC CAG CAA GAT CAA GAC G-3'). PCR was performed for 28 to 32 cycles based on abundance of transcripts of the genes analyzed.

Detection of ROS.

Detection of H₂O₂ and superoxide anion in leaf tissues was performed by 3,3-diaminobenzidine (DAB) staining (Thordal-Christensen et al. 1997) and nitroblue tetrazolium (NBT) staining (Doke et al. 1983), respectively. Leaf samples were collected from inoculated plants 48 h after inoculation. For staining of H₂O₂, individual leaf petioles were dipped into DAB (Sigma) solution (1 mg/ml, pH 3.8) and incubated for 8 h in the dark at room temperature. Thereafter, leaves were removed, placed into acetic acid/glycerol/ethanol (1:1:1, vol/vol/vol), and boiled for 5 min in a water bath. Subsequently, the solution was exchanged and leaves were maintained in 60% glycerol. For staining of superoxide anion, leaves were vacuum infiltrated in 2 ml of 10 mM potassium phosphate buffer, pH 7.5, containing 10 mM NaN₃ and 0.1% NBT (Sigma) for 30 min at room temperature. Leaves were boiled in 96% ethanol for 10 min before visualizing blue precipitates.

ACKNOWLEDGMENTS

We thank the *Arabidopsis* Biological Resource Center at Ohio State University for the T-DNA insertion mutant. This work was supported by National Science Foundation of China (grant no. 30771399), National High-tech Project of China (2007AA10Z140), and National Basic Research Program of China (2006CB101905 and 2009CB119005).

LITERATURE CITED

- Aida, M., Ishida, T., Fukaki, H., Fujisawa, H., and Tasaka, M. 1997. Genes involved in organ separation in *Arabidopsis*: An analysis of the cup-shaped cotyledon mutant. *Plant Cell* 9:841-857.
- Benito, E. P., ten Have, A., van't Klooster, J. W., and van Kan, J. A. L. 1998. Fungal and plant gene expression during synchronized infection of tomato leaves by *Botrytis cinerea*. *Eur. J. Plant Pathol.* 104:207-220.
- Bu, Q., Jiang, H., Li, C.B., Zhai, Q., Zhang, J., Wu, X., Sun, J., Xie, Q., and Li, C. 2008. Role of the *Arabidopsis thaliana* NAC transcription factors ANAC019 and ANAC055 in regulating jasmonic acid-signaled defense responses. *Cell Res.* 18:756-767.
- 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.
- 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.

- Collinge, M., and Boller, T. 2001. Differential induction of two potato genes, *Sprx2* and *StNAC*, in response to infection by *Phytophthora infestans* and to wounding. *Plant Mol. Biol.* 46:521-529.
- Delessert, C., Kazan, K., Wilson, I. W., Van Der Straeten, D., Manners, J., Dennis, E. S., and Dölferus, R. 2005. The transcription factor ATAF2 represses the expression of pathogenesis-related genes in *Arabidopsis*. *Plant J.* 43:745-757.
- de Torres-Zabala, M., Truman, W., Bennett, M. H., Lafforgue, G., Mansfield, J. W., Egea, P. R., Bogre, L., and Grant, M. 2007. *Pseudomonas syringae* pv. *tomato* hijacks the *Arabidopsis* abscisic acid signaling pathway to cause disease. *EMBO (Eur. Mol. Biol. Organ.) J.* 26:1434-1443.
- Doke, N. 1983. Involvement of superoxide anion generation in the hypersensitive response of potato tuber tissue to infection with an incompatible race of *Phytophthora infestans* and to the hyphal wall components. *Physiol. Plant Pathol.* 23:345-357.
- Eulgem, T., and Somssich, I. E. 2007. Networks of WRKY transcription factors in defense signaling. *Curr. Opin. Plant Biol.* 10:366-371.
- Fang, Y., You, J., Xie, K., Xie, W., and Xiong, L. 2008. Systematic sequence analysis and identification of tissue-specific or stress-responsive genes of NAC transcription factor family in rice. *Mol. Genet. Genomics* 280:547-563.
- Ferrari, S., Plotnikova, J. M., De Lorenzo, G., and Ausubel, F. M. 2003. *Arabidopsis* local resistance to *Botrytis cinerea* involves salicylic acid and camalexin and requires EDS4 and PAD2, but not SID2, EDS5 or PAD4. *Plant J.* 35:193-205.
- Fujita, M., Fujita, Y., Maruyama, K., Seki, M., Hiratsu, K., Ohme-Takagi, M., Tran, L. S., Yamaguchi-Shinozaki, K., and Shinozaki, K. 2004. A dehydration-induced NAC protein, RD26, is involved in a novel ABA-dependent stress-signaling pathway. *Plant J.* 39:863-876.
- Glazebrook, J. 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annu. Rev. Phytopathol.* 43:205-227.
- Govrin, E. M., and Levine, A. 2002. Infection of *Arabidopsis* with a necrotrophic pathogen, *Botrytis cinerea*, elicits various defense responses but does not induce systemic acquired resistance (SAR). *Plant Mol. Biol.* 48:267-276.
- Guo, Y., and Gan, S. 2006. AtNAP, a NAC family transcription factor, has an important role in leaf senescence. *Plant J.* 46:601-612.
- Gutterson, N., and Reuber, T. L. 2004. Regulation of disease resistance pathways by AP2/ERF transcription factors. *Curr. Opin. Plant Biol.* 7:465-471.
- He, X. J., Mu, R. L., Cao, W. H., Zhang, Z. G., Zhang, J. S., and Chen, S. Y. 2005. AtNAC2, a transcription factor downstream of ethylene and auxin signaling pathways, is involved in salt stress response and lateral root development. *Plant J.* 44:903-916.
- Hegedus, D., Yu, M., Baldwin, D., Gruber, M., Sharpe, A., Parkin, I., Whitwell, S., and Lydiate, D. 2003. Molecular characterization of *Brassica napus* NAC domain transcriptional activators induced in response to biotic and abiotic stress. *Plant Mol. Biol.* 53:383-397.
- Hiratsu, K., Matsui, K., Koyama, T., and Ohme-Takagi, M. 2003. Dominant repression of target genes by chimeric repressors that include the EAR motif, a repression domain, in *Arabidopsis*. *Plant J.* 34:733-739.
- Hu, H., Dai, M., Yao, J., Xiao, B., Li, X., Zhang, Q., and Xiong, L. 2006. Overexpressing a *NAM*, *ATAF*, and *CUC* (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proc. Natl. Acad. Sci. U.S.A.* 103:12987-12992.
- Hu, H., You, J., Fang, Y., Zhu, X., Qi, Z., and Xiong, L. 2008. Characterization of transcription factor gene *SNAC2* conferring cold and salt tolerance in rice. *Plant Mol. Biol.* 67:169-181.
- Jensen, M. K., Rung, J. H., Gregersen, P. L., Gjetting, T., Fuglsang, A. T., Hansen, M., Joehnk, N., Lyngkjær, M. F., and Collinge, D. B. 2007. The HvNAC6 transcription factor: A positive regulator of penetration resistance in barley and *Arabidopsis*. *Plant Mol. Biol.* 65:137-150.
- Jensen, M. K., Hagedorn, P. H., de Torres-Zabala, M., Grant, M. R., Rung, J. H., Collinge, D. B., and Lyngkjær, M. F. 2008. Transcriptional regulation by a NAC (NAM-ATAF1,2-CUC2) transcription factor attenuates ABA signalling for efficient basal defence towards *Blumeria graminis* f. sp. *hordei* in *Arabidopsis*. *Plant J.* 56:867-880.
- Jeong, R. D., Chandra-Shekar, A. C., Kachroo, A., Klessig, D. F., and Kachroo, P. 2008. HRT-mediated hypersensitive response and resistance to Turnip crinkle virus in *Arabidopsis* does not require the function of TIP, the presumed guard protein. *Mol. Plant-Microbe Interact.* 21:1316-1324.
- Kim, S. G., Lee, A. K., Yoon, H. K., and Park, C. M. 2008. A membrane-bound NAC transcription factor NTL8 regulates gibberellic acid-mediated salt signaling in *Arabidopsis* seed germination. *Plant J.* 55:77-88.
- Kim, Y. S., Kim, S. G., Park, J. E., Park, H. Y., Lim, M. H., Chua, N. H., and Park, C. M. 2006. A membrane-bound NAC transcription factor regulates cell division in *Arabidopsis*. *Plant Cell* 18:3132-3144.
- Lu, P. L., Chen, N. Z., An, R., Su, Z., Qi, B. S., Ren, F., Chen, J., and Wang, X. C. 2007. A novel drought-inducible gene, *ATAF1*, encodes a NAC family protein that negatively regulates the expression of stress-responsive genes in *Arabidopsis*. *Plant Mol. Biol.* 63:289-305.
- Mengiste, T., Chen, X., Salmeron, J., and Dietrich, R. 2003. The *BOTRYTIS SUSCEPTIBLE1* gene encodes an R2R3MYB transcription factor protein that is required for biotic and abiotic stress responses in *Arabidopsis*. *Plant Cell* 15:2551-2565.
- Mitsuda, N., Seki, M., Shinozaki, K., and Ohme-Takagi, M. 2005. The NAC transcription factors NST1 and NST2 of *Arabidopsis* regulate secondary wall thickenings and are required for anther dehiscence. *Plant Cell* 17:2993-3006.
- Mitsuda, N., Iwase, A., Yamamoto, H., Yoshida, M., Seki, M., Shinozaki, K., and Ohme-Takagi, M. 2007. NAC transcription factors, NST1 and NST3, are key regulators of the formation of secondary walls in woody tissues of *Arabidopsis*. *Plant Cell* 19:270-280.
- Mitsuda, N., Umemura, Y., Ikeda, M., Shikata, M., Koyama, T., Matsui, K., Narumi, T., Aida, R., Sasaki, K., Hiyama, T., Higuchi, Y., Ono, M., Isuzugawa, K., Saitoh, K., Endo, R., Ikeda, K., Nakatsuka, T., Nishihara, M., Yamamura, S., Yamamura, T., Terakawa, T., Ohtsubo, N., and Ohme-Takagi, M. 2008. FioreDB: A database of phenotypic information induced by the chimeric repressor silencing technology (CRES-T) in *Arabidopsis* and floricultural plants. *Plant Biotechnol.* 25:37-43.
- Mur, L. A., Kenton, P., Atzorn, R., Miersch, O., and Wasternack, C. 2006. The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. *Plant Physiol.* 140:249-262.
- Nakashima, K., Tran, L. S., Van Nguyen, D., Fujita, M., Maruyama, K., Todaka, D., Ito, Y., Hayashi, N., Shinozaki, K., and Yamaguchi-Shinozaki, K. 2007. Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice. *Plant J.* 51:617-630.
- Ogo, Y., Kobayashi, T., Nakanishi, H., Kakei, Y., Takahashi, M., Toki, S., Mori, S., and Nishizawa, N. K. 2008. A novel NAC transcription factor, IDEF2, that recognizes the iron deficiency-responsive element 2 regulates the genes involved in iron homeostasis in plants. *J. Biol. Chem.* 283:13407-13417.
- Oh, S. K., Lee, S., Yu, S. H., and Choi, D. 2005. Expression of a novel NAC domain-containing transcription factor (CaNAC1) is preferentially associated with incompatible interactions between chili pepper and pathogens. *Planta* 222:876-887.
- Ohnishi, T., Ohnishi, T., Sugahara, S., Yamada, T., Kikuchi, K., Yoshida, Y., Hirano, H. Y., and Tsutsumi, N. 2005. OsNAC6, a member of the NAC gene family, is induced by various stresses in rice. *Genes Genet. Syst.* 80:135-139.
- Olsen, A. N., Ernst, H. A., Leggio, L. L., and Skriver, K. 2005. NAC transcription factors: Structurally distinct, functionally diverse. *Trends Plant Sci.* 10:79-87.
- Ooka, H., Satoh, K., Doi, K., Nagata, T., Otomo, Y., Murakami, K., Matsubara, K., Osato, N., Kawai, J., Carninci, P., Hayashizaki, Y., Suzuki, K., Kojima, K., Takahara, Y., Yamamoto, K., and Kikuchi, S. 2003. Comprehensive analysis of NAC family genes in *Oryza sativa* and *Arabidopsis thaliana*. *DNA Res.* 10:239-247.
- Ren, T., Qu, F., and Morris, T. J. 2000. HRT gene function requires interaction between a NAC protein and viral capsid protein to confer resistance to Turnip crinkle virus. *Plant Cell* 12:1917-1926.
- Sablowski, R. W., and Meyerowitz, E. M. 1998. A homolog of *NO APICAL MERISTEM* is an immediate target of the floral homeotic genes *APETALA3/PISTILLATA*. *Cell* 92:93-103.
- Schenk, P. M., Kazan, K., Wilson, I., Anderson, J. P., Richmond, T., Somerville, S. C., and Manners, J. M. 2000. Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. *Proc. Natl. Acad. Sci. U.S.A.* 97:11655-11660.
- Schenk, P. M., Kazan, K., Manners, J. M., Anderson, J. P., Simpson, R. S., Wilson, I. W., Somerville, S. C., and Maclean, D. J. 2003. Systemic gene expression in *Arabidopsis* during an incompatible interaction with *Alternaria brassicicola*. *Plant Physiol.* 132:999-1010.
- Selth, L. A., Dogra, S. C., Rasheed, M. S., Healy, H., Randles, J. W., and Rezaian, M. A. 2005. A NAC domain protein interacts with tomato leaf curl virus replication accessory protein and enhances viral replication. *Plant Cell* 17:311-325.
- Souer, E., van Houwelingen, A., Kloos, D., Mol, J., and Koes, R. 1996. The *no apical meristem* gene of *Petunia* is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. *Cell* 85:159-170.
- Stein, M., Dittgen, J., Sanchez-Rodriguez, C., Hou, B. H., Molina, A., Schulze-Lefert, P., Lipka, V., and Somerville, S. 2006. *Arabidopsis* PEN3/PDR8, an ATP binding cassette transporter, contributes to non-host resistance to inappropriate pathogens that enter by direct penetration. *Plant Cell* 18:731-746.

- Thordal-Christensen, H., Zhang, Z., Wei, Y., and Collinge, D. B. 1997. Subcellular localization of H₂O₂ in plants. H₂O₂ accumulation in papillae and hypersensitive response during the barley-powdery mildew interaction. *Plant J.* 11:1187-1194.
- Tran, L. S., Nakashima, K., Sakuma, Y., Simpson, S. D., Fujita, Y., Maruyama, K., Fujita, M., Seki, M., Shinozaki, K., and Yamaguchi-Shinozaki, K. 2004. Isolation and functional analysis of *Arabidopsis* stress-inducible NAC transcription factors that bind to a drought-responsive *cis*-element in the early responsive to dehydration stress 1 promoter. *Plant Cell* 16:2481-2498.
- Tran, L. S., Nakashima, K., Sakuma, Y., Osakabe, Y., Qin, F., Simpson, S. D., Maruyama, K., Fujita, Y., Shinozaki, K., and Yamaguchi-Shinozaki, K. 2007. Co-expression of the stress-inducible zinc finger homeodomain ZFHD1 and NAC transcription factors enhances expression of the *ERD1* gene in *Arabidopsis*. *Plant J.* 49:46-63.
- Uauy, C., Distelfeld, A., Fahima, T., Blechl, A., and Dubcovsky, J. 2006. A NAC gene regulating senescence improves grain protein, zinc, and iron content in wheat. *Science* 314:1298-1301.
- Veronese, P., Nakagami, H., Bluhm, B., Abuqamar, S., Chen, X., Salmeron, J., Dietrich, R. A., Hirt, H., and Mengiste, T. 2006. The membrane-anchored BOTRYTIS-INDUCED KINASE1 plays distinct roles in *Arabidopsis* resistance to necrotrophic and biotrophic pathogens. *Plant Cell* 18:257-273.
- Xie, Q., Frugis, G., Colgan, D., and Chua, N. H. 2000. *Arabidopsis* NAC1 transduces auxin signal downstream of TIR1 to promote lateral root development. *Genes Dev.* 14:3024-3036.
- Xing, D.-H., Lai, Z.-B., Zheng, Z.-Y., Vinoda, K. M., Fan, B.-F., and Chen, Z.-X. 2008. Stress- and pathogen-induced *Arabidopsis* WRKY48 is a transcriptional activator that represses plant basal defense. *Mol. Plant* 1:459-470.
- Yoo, S. Y., Kim, Y., Kim, S. Y., Lee, J. S., and Ahn, J. H. 2007. Control of flowering time and cold response by a NAC-domain protein in *Arabidopsis*. *PLoS ONE* 2:e642.
- Yoon, H. K., Kim, S. G., Kim, S. Y., and Park, C. M. 2008. Regulation of leaf senescence by NTL9-mediated osmotic stress signaling in *Arabidopsis*. *Mol. Cells* 25:438-445.
- Yoshii, M., Shimizu, T., Yamazaki, M., Higashi, T., Miyao, A., Hirochika, H., and Omura, T. 2008. Disruption of a novel gene for a NAC-domain protein in rice confers resistance to *Rice dwarf virus*. *Plant J.* 57:615-625.
- Zhang, Z., Feechan, A., Pedersen, C., Newman, M. A., Qiu, J. L., Olesen, K. L., and Thordal-Christensen, H. 2007. A SNARE-protein has opposing functions in penetration resistance and defence signalling pathways. *Plant J.* 49:302-312.
- 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.
- Zheng, X., Chen, B., Lu, G., and Han, B. 2009. Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. *Biochem. Biophys. Res. Commun.* 379:985-989.
- Zhong, R., Demura, T., and Ye, Z. H. 2006. SND1, a NAC domain transcription factor, is a key regulator of secondary wall synthesis in fibers of *Arabidopsis*. *Plant Cell* 18:3158-3170.

AUTHOR-RECOMMENDED INTERNET RESOURCES

FioreDB database: www.cres-t.org/fiore/public_db/f_help.shtml
 Salk Institute Genomic Analysis Laboratory T-DNA Express website, www.signal.salk.edu