The Cysteine2/Histidine2-Type Transcription Factor *ZINC FINGER OF ARABIDOPSIS THALIANA6* Modulates Biotic and Abiotic Stress Responses by Activating Salicylic Acid-Related Genes and *C-REPEAT-BINDING FACTOR* Genes in Arabidopsis[1][C][W]

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The cysteine2/histidine2-type zinc finger proteins are a large family of transcription regulators, and some of them play essential roles in plant responses to biotic and abiotic stress. In this study, we found that expression of C_2H_2-type *ZINC FINGER OF ARABIDOPSIS THALIANA6* (*AtZAT6*) was transcriptionally induced by salt, dehydration, cold stress treatments, and pathogen infection, and AtZAT6 was predominantly located in the nucleus. *AtZAT6*-overexpressing plants exhibited improved resistance to pathogen infection, salt, drought, and freezing stresses, while *AtZAT6* knockdown plants showed decreased stress resistance. AtZAT6 positively modulates expression levels of stress-related genes by directly binding to the TACAAT motifs in the promoter region of pathogen-related genes (*ENHANCED DISEASE SUSCEPTIBILITY1*, *PHYTOALEXIN DEFICIENT4*, *PATHOGENESIS-RELATED GENE1* [*PR1*], *PR2*, and *PR5*) and abiotic stress-responsive genes (*C-REPEAT-BINDING FACTOR1* [*CBF1*], *CBF2*, and *CBF3*). Moreover, overexpression of *AtZAT6* exhibited pleiotrophic phenotypes with curly leaves and small-sized plant at vegetative stage and reduced size of floral organs and siliques at the reproductive stage. Modulation of *AtZAT6* also positively regulates the accumulation of salicylic acid and reactive oxygen species (hydrogen peroxide and superoxide radical). Taken together, our findings indicated that *AtZAT6* plays important roles in plant development and positively modulates biotic and abiotic stress resistance by activating the expression levels of salicylic acid-related genes and *CBF* genes.

In nature, plants live in complex environmental conditions in which various abiotic stresses and multiple microbial pathogens with different infection strategies and lifestyles influence plant growth and development (Bent and Mackey, 2007; Shi et al., 2013a, 2013b). As sessile organisms, plants cannot avoid unfavorable circumstances by adjusting their location. Therefore, they have evolved complex strategies to perceive stress signals and further translate the perception into effective plant responses (Gimenez-Ibanez and Solano, 2013; Shi et al., 2014a, 2014b).

In recent years, much attention has been paid to the roles of the hormones in biotic and abiotic stress responses and especially hormone interactions under stress conditions (Pieterse et al., 2012; Yang et al., 2012a; Liu et al., 2013c). Several plant hormone receptors are located in the nucleus, such as jasmonate and auxin, while the signaling perception of salicylic acid (SA), ethylene, and abscisic acid initiates in the cytoplasm and then translocates to the nucleus. Plant transcription factors (TFs) serve as important mediators during hormone cross talks under biotic and abiotic stress conditions (Kazan and Manners, 2009; Kieffer et al., 2010; Liu et al., 2013c). Currently, various TFs have been shown to be involved in biotic and abiotic stress responses via activating stress-responsive gene expression, such as C-repeat-binding factors (CBFs)/dehydration-responsive element-binding proteins (DREBs), WRKYs, ethylene-responsive element-binding factors, MYBs, MYCs, basic domain-Leu zipper families, and zinc finger proteins (ZFPs; Qiu et al., 2008; Yu et al., 2008; Bi et al., 2010; Li et al., 2010a, 2010b; Seo and Park, 2010; Zhang et al., 2010a, 2010b; Zhu et al., 2010; Cheng et al., 2011).

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ZFPs are a large family of transcription regulators in plants for modulation of downstream stress-responsive genes (Ciftci-Yilmaz et al., 2007; Devaiah et al., 2007; Kodaira et al., 2011; Liu et al., 2013d). ZFPs have been classified into at least nine types based on the number and location of characteristic residues (Cys and His), including C_2H_2, C_2HC, C_2HC_5, C_3H, C_3HC_4, C_4, C_4HC_3, C_6, and C_8 (Nguyen et al., 2012; Bogamuwa and Jang, 2013; Zhou et al., 2013). In Arabidopsis (*Arabidopsis thaliana*), the involvement of C_2H_2-type ZFPs, including *Arabidopsis Zinc-Finger Protein1* (*AZF1*), *AZF2*, *AZF3*, *Drought induced19* (*Di19*), *Zinc Finger of Arabidopsis6* (*ZAT6*), *ZAT7*, *ZAT10*, and *ZAT12*, in plant abiotic stress response has been revealed (Liu et al., 2013b, 2013d). Kodaira et al. (2011) found that *AtAZF1* and *AtZAF2* negatively regulated abscisic acid-repressive and auxin-inducible genes under abiotic stress conditions. Constitutive expression of *AtZAT12* conferred improved resistance to high light and osmotic and oxidative stresses, whereas knockout plants of *AtZAT12* exhibited increased sensitivity to osmotic and salt stresses (Rizhsky et al., 2004; Davletova et al., 2005). *AtZAT10* played a dual role in response to abiotic stress because both gain- and loss-of-function mutations of *AtZAT10* displayed enhanced resistance to drought and osmotic and salt stresses (Mittler et al., 2006). Recently, AtZAT10 was identified as a substrate of mitogen-activated protein kinase (Nguyen et al., 2012). Ciftci-Yilmaz et al. (2007) revealed that *AtZAT7* positively mediated salt stress tolerance via modulating expression of several defense-responsive genes and that the ethylene-responsive element-binding factor-associated amphiphilic repression motif is required for abiotic stress response. *AtZAT6* is a repressor of primary root growth that modulates phosphate homeostasis through the control of root architecture (Devaiah et al., 2007). Moreover, AtZAT6 was identified as a novel target of MITOGEN-ACTIVATED PROTEIN KINASE6 (MPK6), and phosphorylation of AtZAT6 is essential for its positive regulation of seed germination under salt and osmotic stresses (Liu et al., 2013d). Overexpressing chimeric repressors derived *AtZAT6* and also conferred enhanced salt tolerance (Mito et al., 2011). However, the molecular mechanisms of ZAT6 in plant response to abiotic stress remain unclear, and the possible role of ZAT6 in plant-pathogen interaction remains unknown.

In this study, we found that *AtZAT6* positively regulated biotic and abiotic stress resistances, and overexpression of *AtZAT6* resulted in distinct developmental phenotypes. Additionally, the regulatory mechanism underlying *AtZAT6*-mediated stress responses was also characterized. The results indicated that *AtZAT6* plays important

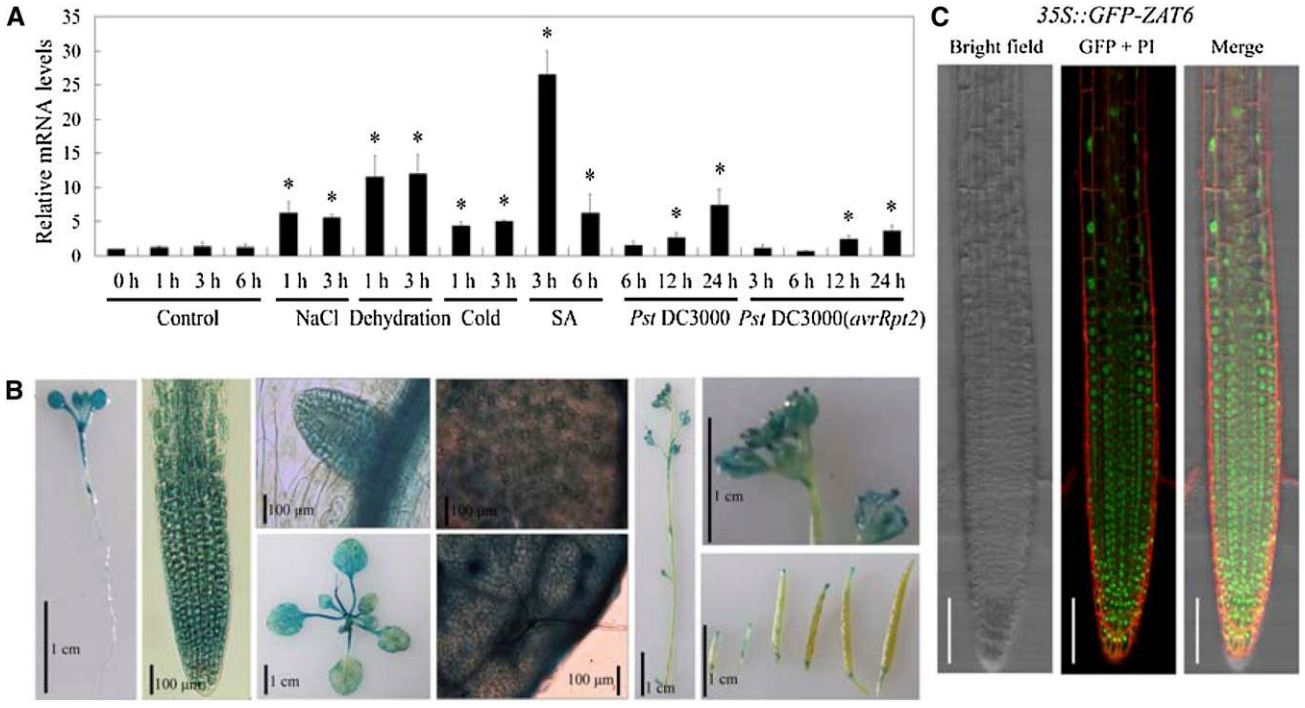

Figure 1. The expression pattern of *AtZAT6*. A, The expression levels of *AtZAT6* after stress treatments. For real-time PCR, 28-d-old Col-0 plants were treated by 300 mM NaCl, dehydration, 4°C, 100 μM SA, *Pst* DC3000, and *Pst* DC3000(*avrRpt2*) for designed times. The expression level of *AtZAT6* at 0 h of treatment was normalized as 1.0. The results shown are the means ± SDs of at least three independent experiments. Asterisks indicate the significant difference of $P < 0.05$ compared with the wild type under control condition for 0 h. B, GUS staining of *ProZAT6::GUS* transgenic plants in different organs. C, Subcellular localization of AtZAT6. GFP signals were detected in 5-d-old seedling roots of *35S::GFP-ZAT6* transgenic plants. The red fluorescence indicates cell wall by propidium iodide staining, and the green fluorescence indicates the localization of AtZAT6. Bars = 100 μm. [See online article for color version of this figure.]

roles in stress responses through activating the expression levels of SA-related genes and *CBF* genes.

RESULTS

Expression Pattern of *AtZAT6* under Stress Treatments

Based on publicly available gene expression data analysis (Winter et al., 2007), we found that *AtZAT6* was highly induced by most abiotic and biotic stress treatments (Supplemental Fig. S1). The expression pattern of *AtZAT6* was further examined through real-time PCR. Consistent with microarray data, infections with *Pseudomonas syringae* pv *tomato* (*Pst*) DC3000 and *Pst* DC3000 (harboring the avirulence gene of *Rpt2* [*avrRpt2*]) significantly induced the transcript level of *AtZAT6* in a time-dependent manner (Fig. 1A). In addition, expression level of *AtZAT6* was also transcriptionally induced after abiotic stress treatments, including salt, dehydration, and cold stresses (Fig. 1A). Interestingly, the transcript level of *AtZAT6* was largely induced by SA treatment (Fig. 1A). These results indicated that *AtZAT6* might play some roles in plant responses to biotic stress and abiotic stress.

Using *ProZAT6*::*GUS* transgenic plants, *AtZAT6* was found to be strongly expressed in cotyledons, leaves, and roots, but weakly in flowers, siliques, and stems (Fig. 1B). In the transgenic *35S*::*GFP-ZAT6* plants, GFP signals were detected in 5-d-old seedling roots. As shown in Figure 1C, *35S*::*GFP-ZAT6* fluorescence was predominantly localized in the nucleus and partially in the cytosol. This result indicated that AtZAT6 is a nuclear protein, which is consistent with the results of Devaiah et al. (2007). Additionally, yeast (*Saccharomyces cerevisiae*) transformation assay showed that AtZAT6 had transactivation activity in yeast (Supplemental Fig. S2).

AtZAT6 Regulates Plant Growth and Development

To reveal the in vivo role of *AtZAT6*, we constructed *AtZAT6*-overexpressing and knockdown transformations in the background of Columbia (Col-0) Arabidopsis plants. After selection by kanamycin and Basta

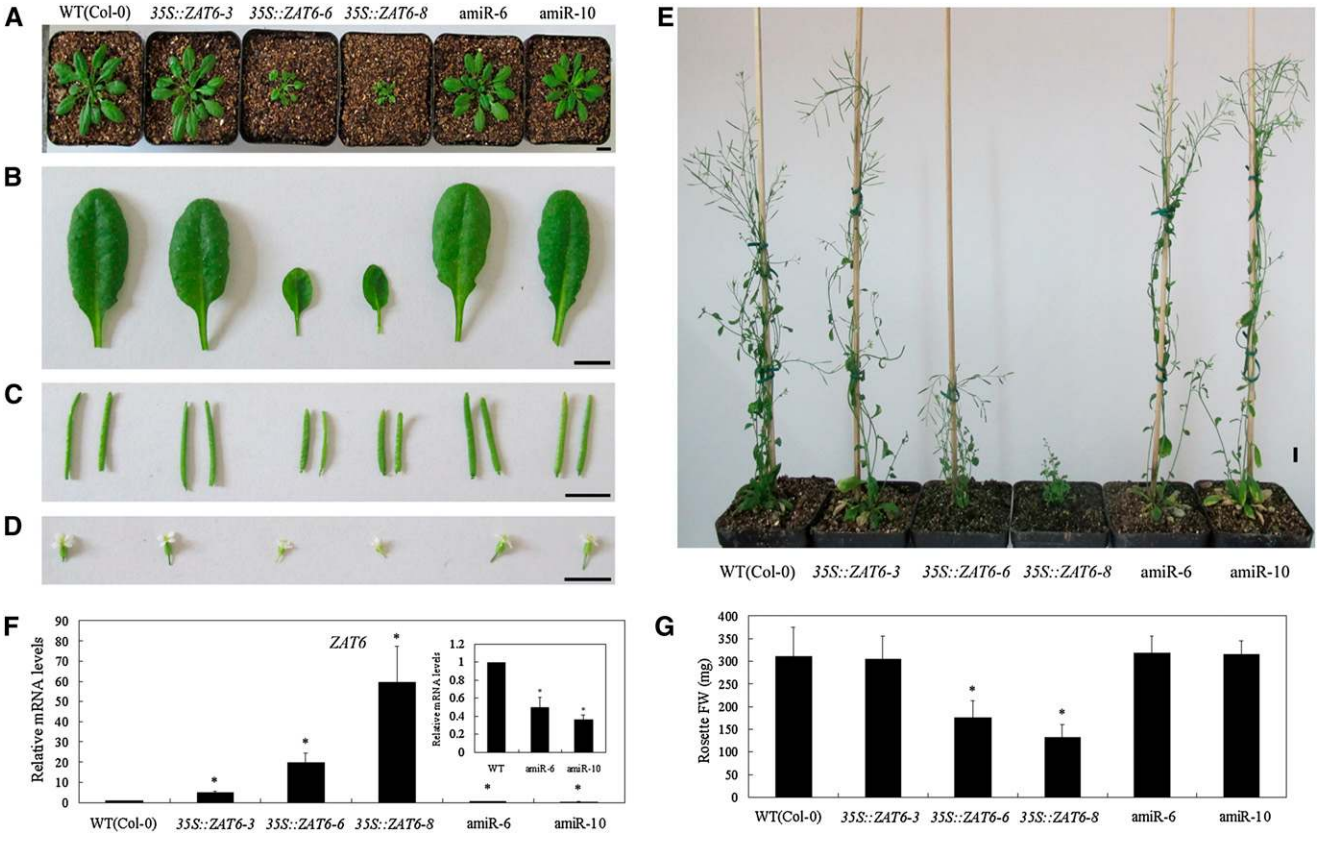

Figure 2. Developmental phenotypes of *AtZAT6*-overexpressing and knockdown plants. A, Twenty-eight-day-old plants in soil. B to D, Size of leaves (B), siliques (C), and floral organs (D) in wild-type (WT) and *AtZAT6*-overexpressing and knockdown plants. Bars = 1 cm. E, Seventy-day-old wild-type and *AtZAT6*-overexpressing and knockdown plants. F, *AtZAT6* expression levels in wild-type and *AtZAT6*-overexpressing and knockdown plants. The expression level of *AtZAT6* in the wild type was normalized as 1.0 ($n = 3$). G, Fresh weight per plant of 28-d-old wild-type and *AtZAT6*-overexpressing and knockdown plants. Means ± SDS ($n = 15$) are shown, and asterisks indicate the significant difference of $P < 0.05$ compared with the wild type. [See online article for color version of this figure.]

resistance, the expression levels of *AtZAT6* in the T1 generation of *35S::ZAT6* and artificial microRNA (amiR)-ZAT6 transgenic plants were verified by real-time PCR (Supplemental Figs. S3 and S4). The T1 generation of *AtZAT6* transgenic plants exhibited pleiotropic developmental phenotypes, including retarded growth with small and curly leaves, and several lines with relatively high transgene expression (lines 5, 10, and 11 with 243.42-, 504.04-, and 337.85-fold relative to the wild type [Col-0], respectively) could not

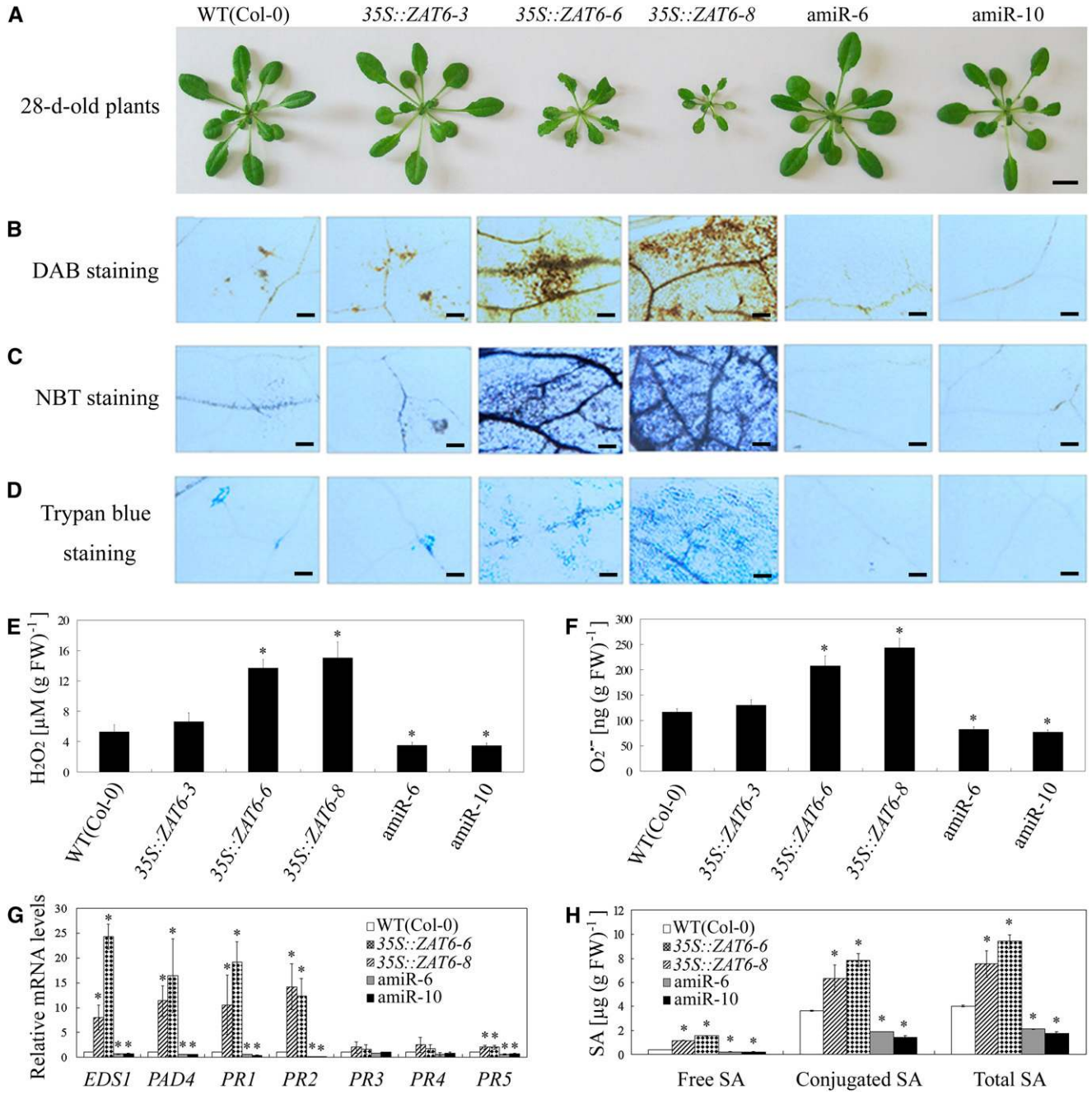

Figure 3. Modulation of *AtZAT6* expression affects defense responses. A, Morphology of 28-d-old soil-grown wild-type (WT) and *AtZAT6*-overexpressing and knockdown plants. Bars = 1 cm. B to D, DAB staining (B), NBT staining (C), and trypan blue staining (D) of 28-d-old soil-grown wild-type and *AtZAT6*-overexpressing and knockdown plants. Bars = 100 μm. E and F, Quantification of H_2O_2 content (E) and $O_2\bullet^-$ content (F). G, Pathogen-related genes' expression in wild-type and *AtZAT6*-overexpressing and knockdown plants. H, The accumulation of free SA, conjugated SA, and total SA assayed by HPLC. The results shown are the means ± SDs of at least three independent experiments. Asterisks indicate the significant difference of $P < 0.05$ compared with the wild type. [See online article for color version of this figure.]

flower and set seeds (Supplemental Fig. S3). Consistently, T2 and T3 generations of *AtZAT6* transgenic plants (lines 6 and 8) with relatively low transgene expression showed similar growth to those of the T1 generation, further confirming the pleiotropic developmental phenotypes of *AtZAT6*-overexpressing plants (Fig. 2A). It has been reported that RNA interference suppression of *AtZAT6* appears to be lethal (Devaiah et al., 2007). In our study, we also found that *AtZAT6* knockdown lines with relatively lower *AtZAT6* transcripts could not flower and set seeds. Therefore, two *AtZAT6* knockdown lines (lines 6 and 10) with about 40% expression level of the wild type were used for further study (Fig. 2A).

When grown on Murashige and Skoog (MS) medium, *AtZAT6*-overexpressing plants showed significantly shorter primary roots and displayed abnormal cotyledons (Supplemental Fig. S5, A–C). At the vegetative stage on soil, *AtZAT6*-overexpressing plants (lines 6 and 8) exhibited retarded growth with bleached leaves, but line 3 with the lowest (6.4-fold) *AtZAT6* overexpression level and *AtZAT6* knockdown plants showed no significant difference with the wild type (Fig. 2, A, B, F, and G). At the productive stage, *AtZAT6*-overexpressing plants (lines 6 and 8) exhibited significantly retarded growth, with a greater rosette leaf number, lower plant height, lesser cauline branch number, smaller leaf length and width, and reduced size of siliques and floral organs, including sepals, petals, stamens, and carpels (Fig. 2, B–E; Supplemental Table S1). However, *AtZAT6* knockdown plants (lines 6 and 10) displayed no significant difference with wild-type plants for the whole growth stage (Fig. 2, B–E; Supplemental Table S1). All of these results provide genetic evidence that *AtZAT6* is involved in plant growth and development.

AtZAT6 Positively Regulates Defense Resistance to Bacterial Pathogen Infection

As shown in Figure 3A, *AtZAT6*-overexpressing plants (lines 6 and 8) exhibited dwarf morphology, whereas overexpressing line 3 and *AtZAT6* knockdown plants (lines 6 and 10) exhibited wild-type-like morphology. Diaminobenzidine (DAB) staining and nitro blue tetrazolium (NBT) staining showed that the *AtZAT6*-overexpressing plants accumulated higher levels of hydrogen peroxide (H_2O_2) and superoxide radical ($O_2\bullet^-$) than those in the wild type, while *AtZAT6* knockdown plants accumulated lower H_2O_2 and $O_2\bullet^-$ contents (Fig. 3, B and C). Quantification of H_2O_2 and $O_2\bullet^-$ contents in leaves of 28-d-old soil-grown *AtZAT6*-overexpressing and knockdown plants also confirmed the results of DAB and NBT staining (Fig. 3,

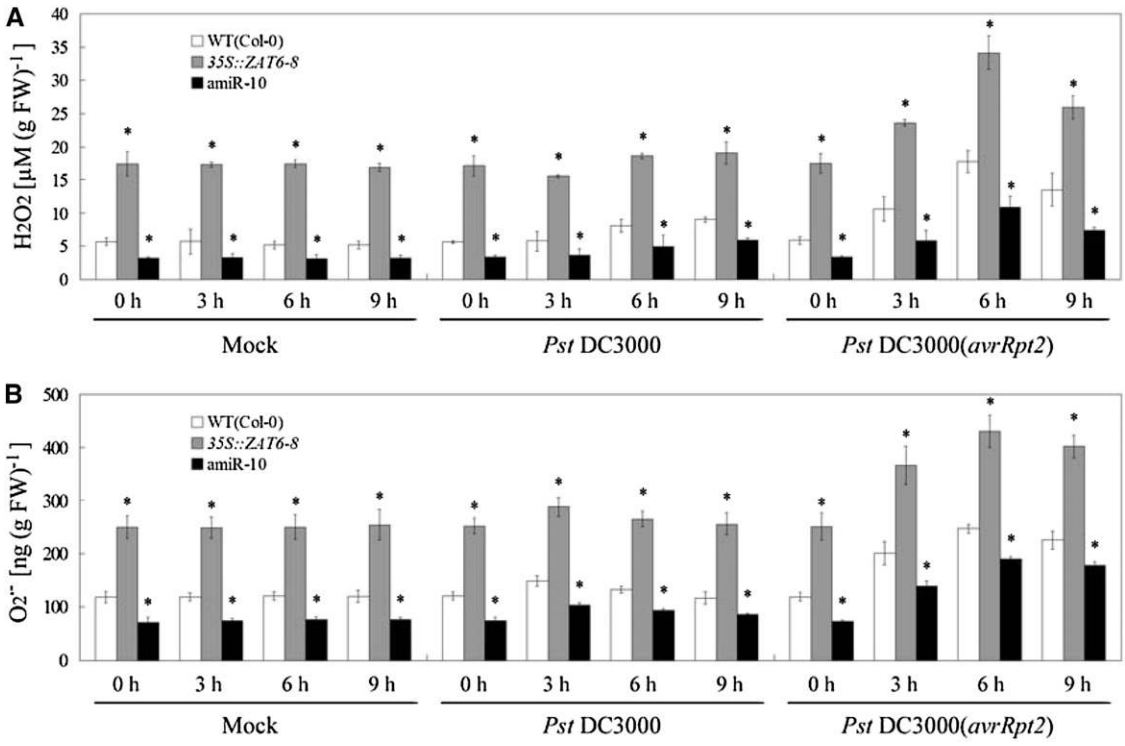

Figure 4. Modulation of *AtZAT6* expression affects oxidative burst under mock and pathogen-infected conditions. A and B, Quantification of H_2O_2 content (A) and $O_2\bullet^-$ content (B) in plants under mock and pathogen-infected conditions. For the assays, 28-d-old plant leaves were infected with mock (10 mM $MgCl_2$), *Pst* DC3000 plus 10 mM $MgCl_2$, and *Pst* DC3000(*avrRpt2*) plus 10 mM $MgCl_2$ for designed times. Means ± SDs of three independent experiments are shown in the results, and asterisks indicate the significant difference of $P < 0.05$ compared with the wild type (WT).

E and F). Trypan blue staining showed that there was extensive cell death in the *AtZAT6*-overexpressing plants, with little in the *AtZAT6* knockdown plants (Fig. 3D). The glutathione antioxidant pool and redox state were also modulated by *AtZAT6* (Supplemental Fig. S6, A–C). *AtZAT6*-overexpressing plants accumulated lower reduced glutathione (GSH) and higher oxidized glutathione (GSSG), while *AtZAT6* knockdown plants displayed increased GSH and decreased GSSG (Supplemental Fig. S6, A–C). In addition, some pathogen-related marker genes, including *ENHANCED DISEASE SUSCEPTIBILITY1* (*EDS1*), *PHYTOALEXIN DEFICIENT4* (*PAD4*), *PATHOGENESIS-RELATED GENE1* (*PR1*), *PR2*, and *PR5*, were constitutively expressed in the *AtZAT6*-overexpressing plants but exhibited lower mRNA levels in the *AtZAT6* knockdown plants (Fig. 3G). Moreover, free and conjugated SA contents were also much higher in the overexpressing transgenic plants but were lower in the *AtZAT6* knockdown plants than those of wild-type plants (Fig. 3H). These data indicated that cell death and defense responses were constitutively activated in *AtZAT6*-overexpressing plants but were repressed in *AtZAT6* knockdown plants.

As reviewed by Lamb and Dixon (1997), oxidative burst and associated changes in defense gene expression following bacterial recognition occurs about 3 to 10 h after inoculation. Then, we examined the effects of *AtZAT6* expression on oxidative burst and associated changes in defense gene expression at early periods after inoculations by mock, *Pst* DC3000, and *Pst* DC3000(*avrRpt2*). During the period between 0 and 9 h post inoculation (hpi) of *Pst* DC3000(*avrRpt2*), oxidative burst, including the accumulations of H_2O_2 and $O_2\bullet^-$, was significantly displayed in wild-type plants (Fig. 4, A and B). Compared with wild-type plants, *AtZAT6*-overexpressing plants exhibited higher levels of H_2O_2 and $O_2\bullet^-$ at 3, 6, and 9 hpi of *Pst* DC3000 (*avrRpt2*), while *AtZAT6* knockdown plants displayed relatively lower levels (Fig. 4, A and B). After inoculation of *Pst* DC3000, the H_2O_2 content was only slightly induced at 6 and 9 hpi in wild-type plants, while the $O_2\bullet^-$ content was not significantly regulated (Fig. 4, A and B). During the period between 0 and 9 hpi of *Pst* DC3000, *AtZAT6*-overexpressing plants exhibited higher levels of H_2O_2 and $O_2\bullet^-$ than those in wild-type plants, while *AtZAT6* knockdown plants displayed relatively lower levels (Fig. 4, A and B).

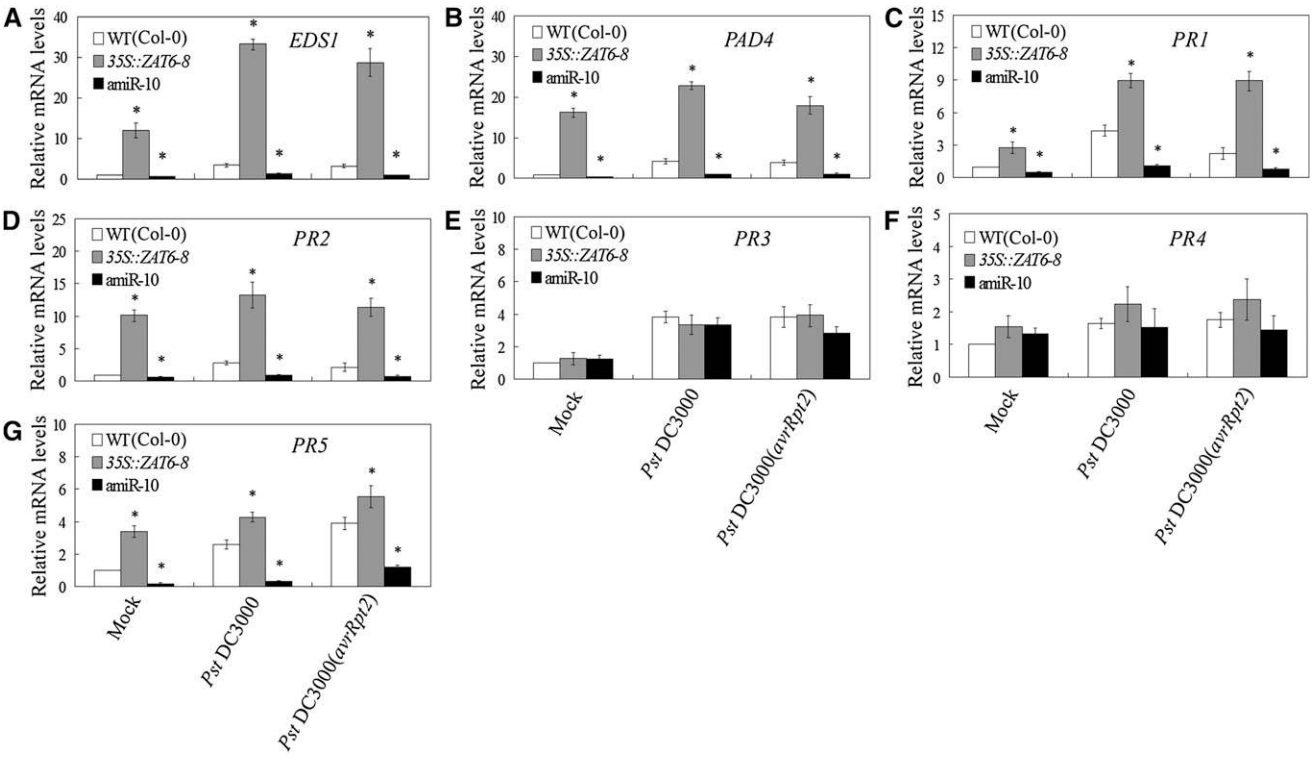

Figure 5. Modulation of *AtZAT6* expression affects defense gene expression under mock and pathogen-infected conditions. A to G, Expression analysis of pathogen-related genes in wild-type (WT) and *AtZAT6*-overexpressing and knockdown plants. The expression levels of *EDS1* (A), *PAD4* (B), *PR1* (C), *PR2* (D), *PR3* (E), *PR4* (F), and *PR5* (G) in plants after mock and pathogen infection for 6 h. The expression level of each gene in wild-type plants by mock infection was normalized as 1.0. For the assays, 28-d-old plant leaves were infected with mock (10 mM $MgCl_2$), *Pst* DC3000 plus 10 mM $MgCl_2$, and *Pst* DC3000(*avrRpt2*) plus 10 mM $MgCl_2$ for 6 h. Means ± SDs of three independent experiments are shown in the results, and asterisks indicate the significant difference of $P < 0.05$ compared with the wild type.

After pathogen infection for 6 h, the expression levels of *EDS1*, *PAD4*, *PR1*, *PR2*, *PR3*, *PR4*, and *PR5* were transcriptionally induced compared with those of mock treatment; however, *AtZAT6*-overexpressing plants exhibited higher transcripts of *EDS1*, *PAD4*, *PR1*, *PR2*, and *PR5* than those in the wild type, while *AtZAT6* knockdown plants displayed relatively lower levels (Fig. 5, A–G). These results indicated that *AtZAT6* positively modulated reactive oxygen species (ROS) level and SA-related gene expressions under both control (mock) and pathogen-infected conditions.

To test whether *AtZAT6* regulates disease resistance against pathogenic bacteria, we examined the basal resistance and systemic acquired resistance (SAR) to *Pst* DC3000 and *Pst* DC3000(*avrRpt2*) in the *AtZAT6*-overexpressing and knockdown plants. Quantification of bacteria number in the pathogen-infected Arabidopsis leaves showed that the *AtZAT6*-overexpressing plants exhibited significantly less bacterial propagation than the wild type at 3 d post infection (dpi) of these pathogens, while the *AtZAT6* knockdown plants exhibited significantly more bacterial propagation than the wild type (Fig. 6, A–C). Therefore, these results indicated that *AtZAT6* positively regulated basal resistance and SAR against pathogenic bacteria.

AtZAT6 Positively Modulates Abiotic Stress Resistance

Because the expression of *AtZAT6* was transcriptionally induced by multiple abiotic stresses, including salt, dehydration, and cold (Fig. 1A; Supplemental Fig. S1), the involvement of *AtZAT6* in abiotic stress resistance was also dissected using *AtZAT6*-overexpressing and knockdown plants. After salt, drought, and chilling stress treatments, *AtZAT6*-overexpressing plants showed better growth with higher survival rate than the wild type and conferred improved resistance to abiotic stresses, while *AtZAT6* knockdown plants exhibited severely inhibited growth with lower survival rate than the wild type (Fig. 7, A and B). Additionally, the expression levels of *CBF1*, *CBF2*, and *CBF3* were constitutively expressed in the *AtZAT6*-overexpressing plants but exhibited lower mRNA levels in the *AtZAT6* knockdown plants (Fig. 7C). However, modulation of *AtZAT6* had no significant effect on the expression of *CBF4*, *DREB2A*, and *DREB2B* (Fig. 7C).

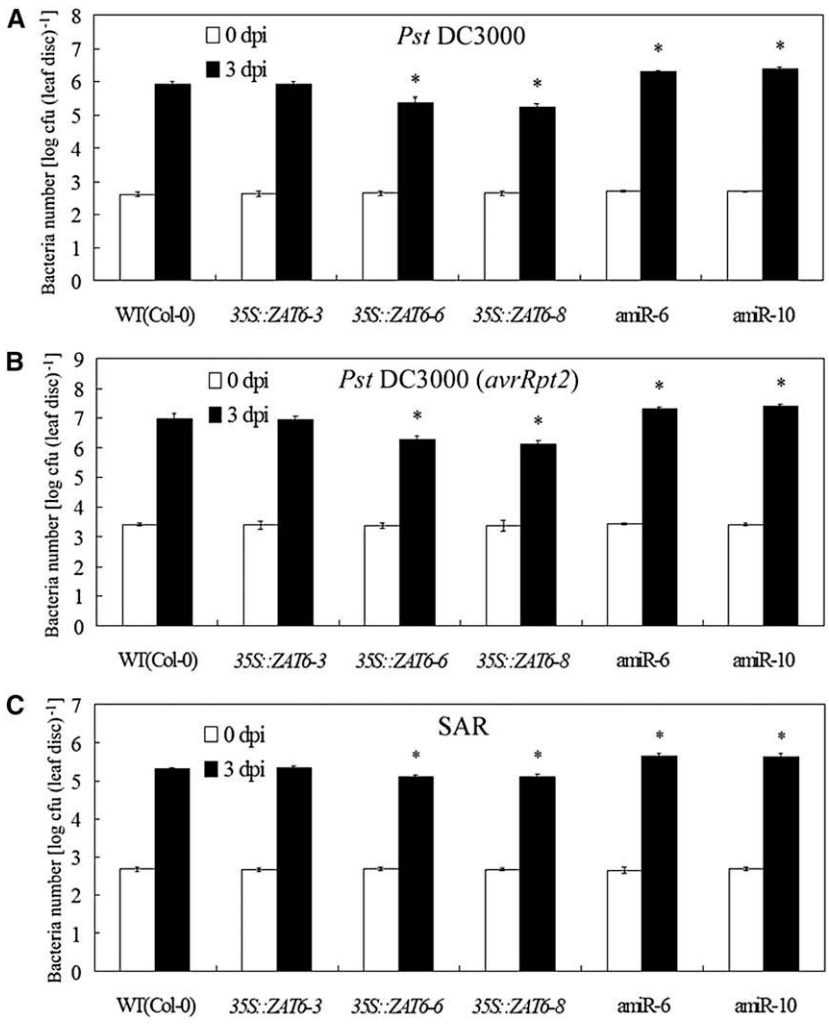

Figure 6. Modulation of *AtZAT6* expression affects disease resistance against bacterial infection. A and B, Growth of *Pst* DC3000 (A) and *Pst* DC3000(*avrRpt2*; B) on plants at 0 and 3 dpi of bacterial infection. C, SAR assay of growth of *Pst* DC3000 on plants at 0 and 3 dpi of bacterial infection. The results shown are the average means ± SDs of four independent experiments, and 20 independent leaf discs were harvested in each independent experiment. Asterisks indicate the significant difference of $P < 0.05$ compared with the wild type (WT).

These results indicated that *AtZAT6* positively modulated abiotic stress resistance and the expressions of CBF genes (*CBF1*, *CBF2*, and *CBF3*).

AtZAT6 Can Bind to the TACAAT Motif of Several Pathogen-Related Genes and *CBF* Genes

To reveal how *ATZAT6* modulated the expressions of the pathogen-related genes and *CBF* genes, we analyzed the promoter sequences of these genes, and the results showed that TACAAT motifs were enriched (Fig. 8C). Liu et al. (2013b) found that AtDi19 (one C_2H_2 ZFP) could modulate the expression levels of *PR1*, *PR2*, and *PR5* through binding to the TACAAT elements within the *PR1*, *PR2*, and *PR5* promoters; thus, whether AtZAT6 could interact with the TACAAT element was investigated in this study. Then, transient expression assays in tobacco (*Nicotiana tabacum*) leaves were performed using 35S-vector and 35S-AtZAT6 as the effectors and m35S-GUS, TACAAT-m35S-GUS, and TAAAAT-m35S-GUS as the reporters (Fig. 8A). As shown in Figure 8B, only the leaf pieces cotransformed with 35S-AtZAT6, and TACAAT-m35S-GUS largely activated the expression of GUS, indicating the in vivo interaction of AtZAT6 and the TACAAT motif. To further investigate the interaction of AtZAT6 and the promoters of several pathogen-related genes, chromatin immunoprecipitation (ChIP)-PCR was performed using *35S*::*GFP-ZAT6-22* transgenic plants, which had the similar phenotype with AtZAT6-overexpressing plants (Supplemental Fig. S7, A–D). The results showed that the AtZAT6 protein strongly interacted with several fragments in the promoters of *EDS1*, *PAD4*, *PR1*, *PR2*, *PR5*, *CBF1*, *CBF2*, and *CBF3* that contained the

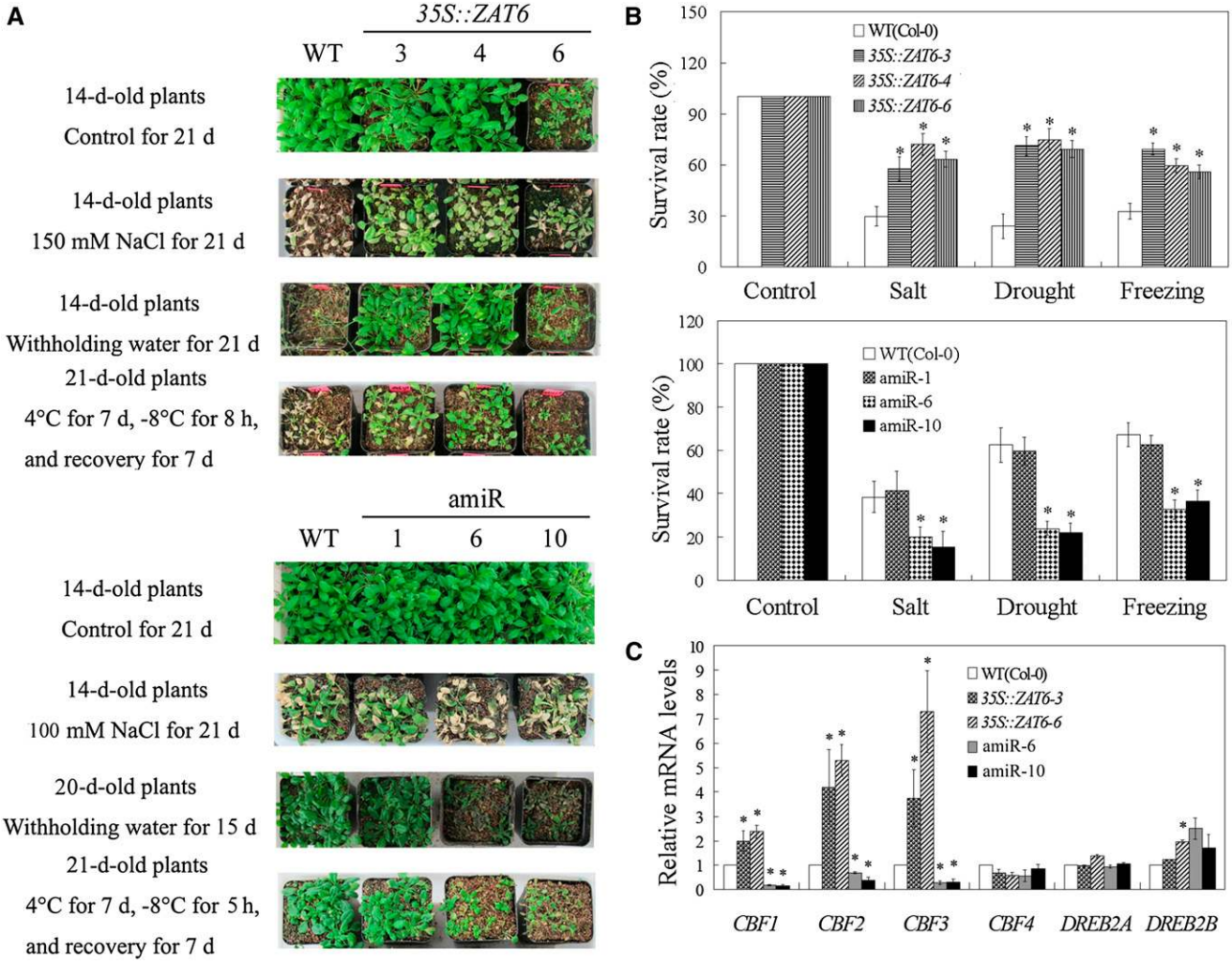

Figure 7. Modulation of *AtZAT6* expression affects abiotic stress resistance. A, Phenotype of plants after abiotic stress treatments for designed days. B, Survival rate of the wild type (WT) and different *AtZAT6* lines at 7 d after recovery from abiotic stress treatments. C, The expression of *CBF* genes in wild-type and *AtZAT6*-overexpressing and knockdown plants. Means ± SDs of at least three independent experiments are shown in the results, and asterisks indicate the significant difference of $P < 0.05$ compared with the wild type. [See online article for color version of this figure.]

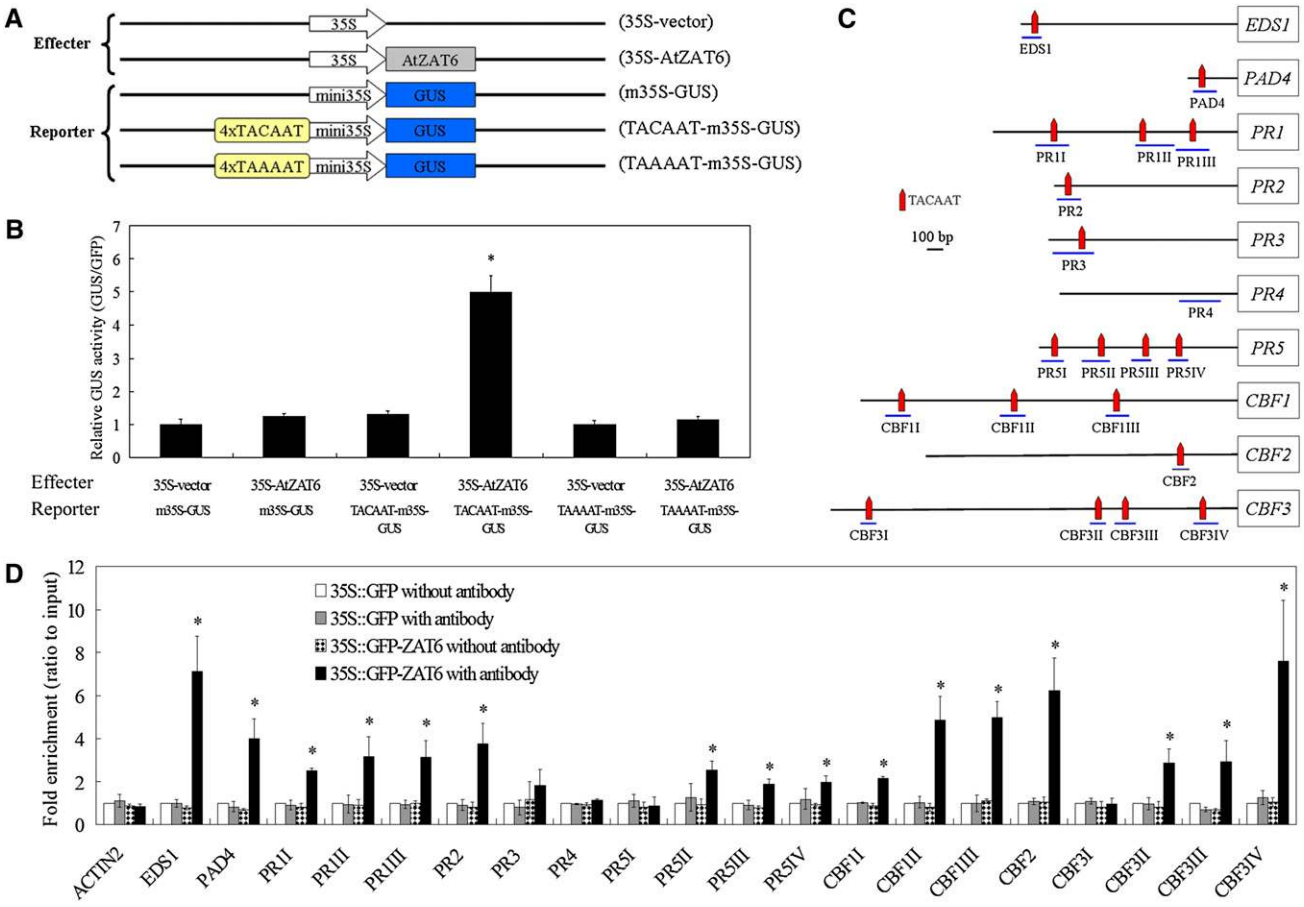

Figure 8. AtZAT6 binds to the promoters of pathogen-related genes and *CBF* genes. A, Schematic diagrams of the effectors (35S-vector and 35S-AtZAT6) and reporters (m35S-GUS, TACAAT-m35S-GUS, and TAAAAT-m35S-GUS) used for transient expression analysis. B, Transient expression of representative tobacco leaves infiltrated with different combinations of effector and reporter. *35S::GFP* plastid was coinjected as an internal standard in each infection. C, Schematic structure of the TACAAT motifs in the promoter of pathogen-related genes and *CBF* genes. The relative positions were chosen for ChIP-PCR analysis. D, ChIP enrichment to show the in vivo binding ability of *35S::GFP-ZAT6* to the DNA fragments in the promoters of pathogen-related genes and *CBF* genes. The ChIP results were normalized to input chromatin, and a fragment in the *ACTIN2* promoter was used as the negative control. The data represent the means ± SDs of three independent experiments, and asterisks indicate the significant difference of $P < 0.05$. [See online article for color version of this figure.]

TACAAT motif but could not interact with the fragments in the promoters of *ACTIN2* and *PR4* (Fig. 8, C and D), indicating that the TACAAT motif is important for the binding of AtZAT6 to regulate the expressions of pathogen-related genes and *CBF* genes.

DISCUSSION

Plant hormones play essential roles in integrating developmental and defense cues into complex signaling networks that not only establish plant developmental architecture, but also build up plant defense responses to biotic and abiotic stresses (Kazan and Manners, 2009; Seo and Park, 2010; Liu et al., 2013c). Among these hormones, SA serves as the most important defense hormone associated with microbial pathogenesis (Kieffer et al., 2010; Xu et al., 2011; Zhang et al., 2012).

In this study, a novel function for *AtZAT6* in plant development and biotic and abiotic stress responses has been elucidated based on phenotypic, molecular, and physiological characterizations. We found that *AtZAT6*-overexpressing plants exhibited constitutively activated defense responses, with increased levels of ROS accumulation (H_2O_2 and $O_2^{\bullet-}$) and extensive cell death in the leaves, while *AtZAT6* knockdown plants showed decreased defense responses, with lower ROS accumulation and less cell death (Fig. 3, B–F). In accordance with other studies, *Pst* DC3000(*avrRpt2*) triggered ROS production in wild-type plants at the period of 3 to 9 hpi because of the recognition of *avrRpt2* by the Resistance to *P. syringae2* resistance protein (Kunkel et al., 1993; Lamb and Dixon, 1997). Moreover, *AtZAT6*-overexpressing plants exhibited even higher levels, while *AtZAT6* knockdown plants displayed relatively lower levels of H_2O_2 and $O_2^{\bullet-}$ at these periods

after inoculation of *Pst* DC3000(*avrRpt2*; Fig. 4, A and B). The rapid induction of ROS by *Pst* DC3000(*avrRpt2*) and *AtZAT6*-mediated ROS accumulation after infection with *Pst* DC3000(*avrRpt2*) might be largely contributed to hypersensitive response (Kunkel et al., 1993; Lamb and Dixon, 1997). However, *Pst* DC3000 only slightly induced the H_2O_2 content at 6 and 9 hpi but had no significant effect on the $O_2\bullet^-$ content at the period of 3 to 9 hpi. Additionally, *AtZAT6*-overexpressing plants exhibited higher levels of H_2O_2 and $O_2\bullet^-$ at the period of 3 to 9 hpi of *Pst* DC3000, while *AtZAT6* knockdown plants displayed relatively lower levels than those in wild-type plants (Fig. 4, A and B). Thus, *AtZAT6* positively modulates ROS level under both mock and pathogen-infected conditions (Fig. 4, A and B), indicating putative links between *AtZAT6*-mediated ROS accumulation and disease resistance. Miller et al. (2008) have reviewed that AtZAT proteins (AtZAT7, AtZAT10, and AtZAT12) are key regulators of ROS signaling and could function as integrators of ROS signaling and plant stress responses. Our study indicated that AtZAT6 is another important factor of the ZAT network-involved ROS signaling. As Miller et al. (2008) reviewed, further studies are needed to investigate how ZAT proteins mediate ROS signaling.

Moreover, the dwarf and retarded phenotype of *AtZAT6*-overexpressing plants is similar to reported dwarf mutants that displayed constitutive activation of ROS and SA-dependent responses, such as activation-tagged mutant of *GH3.5* (Zhang et al., 2007), *myb96-1d* (Seo and Park, 2010), *snc2-1D* (for suppressor of *npr1-1* [knockout mutant of *NON-INDUCIBLE IMMUNITY1, NONEXPRESSER OF PR GENES1*], constitutive2; Zhang et al., 2010b), *snc1* and *Toll-like/interleukin-1 receptor1-human influenza virus hemagglutinin* (Zhu et al., 2010), loss-of-function of *CULLIN1* (Cheng et al., 2011), *mos14-1 snc1 npr1* containing the *MOS14* [*modifier of snc1-1, 14*] transgene under its native promoter; Xu et al., 2011), *mpk4* (Kong et al., 2012), loss-of-function mutant of *BDA1* (for *bian da*; *becoming big* in Chinese; Yang et al., 2012b), *mkk1/mkk2* for mutant of *MAP kinase kinase1* [*MKK1*]/*MKK2*; Zhang et al., 2012), and mutant of *BAK1-INTERACTING RECEPTOR-LIKE KINASE1* (Liu et al., 2013a). Additionally, the expression of *EDS1* and *PAD4*, which are responsible for SA biosynthesis, were significantly up-regulated after overexpression of *AtZAT6* (Fig. 3G). SA accumulation is necessary for the activation of SA downstream gene expression (Zhang et al., 2010b, 2012; Zhu et al., 2010; Kong et al., 2012). Consistently, *AtZAT6*-overexpressing plants showed increased endogenous SA and up-regulated expression of SA-dependent *PR* genes, resulting in enhanced immunity resistance against pathogen bacterials, while *AtZAT6* knockdown plants exhibited decreased immunity resistance (Fig. 3). Similar to AtDi19 (one C_2H_2 ZFP; Liu et al., 2013b), AtZAT6 can bind to the TACAAT elements within the *PR1*, *PR2*, and *PR5* promoters, as well as the TACAAT elements in the *EDS1* and *PAD4* promoters (Fig. 8). Thus, *AtZAT6* positively regulates endogenous SA and the expression of SA-dependent *PR* genes via directly binding to the TACAAT elements of pathogen-related genes.

In addition to biotic stress, *AtZAT6* positively modulated abiotic stress resistance, including salt, drought, and cold stresses. Liu et al. (2013d) found that phosphorylation of AtZAT6 by AtMPK6 was essential for its positive regulation of seed germination under salt and osmotic stresses (Liu et al., 2013d). According to Liu et al. (2013b), the expressions of *PR1*, *PR2*, and *PR5* were largely induced by drought stress treatment. Overexpression of these *PR* genes and pretreatment with SA analogs 2,6-dichloroisonicotinic acid conferred

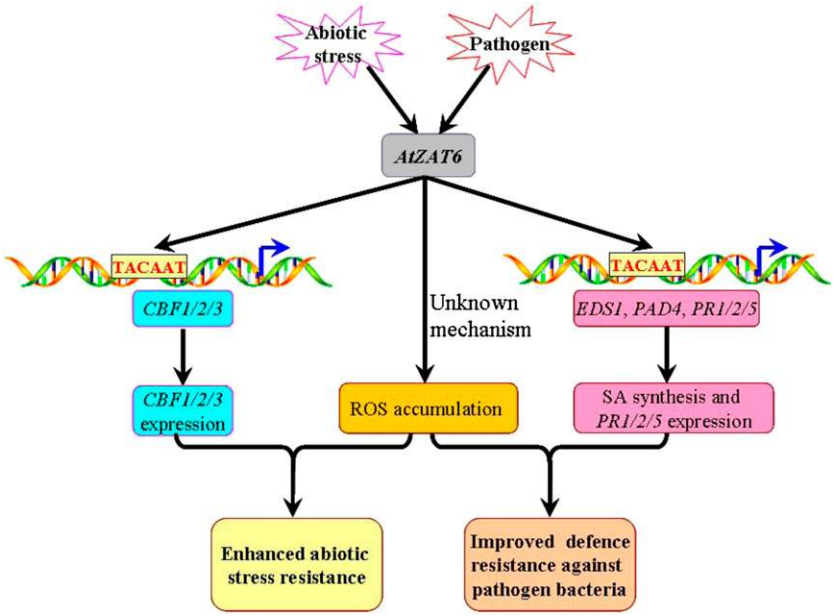

Figure 9. Model depicting the mechanisms of *AtZAT6* involved in plant stress responses. Pathogen and abiotic stress could largely induce the expression of *AtZAT6*. First, *AtZAT6* could bind to the TACAAT elements of the promoters of *EDS1*, *PAD4*, *PR1*/*PR2*/*PR5*, and *CBF1*/*CBF2*/*CBF3*, leading to accumulation of SA and up-regulation of these genes' expression. Second, *AtZAT6*-overexpressing plants could result in higher ROS concentration. All of these results resulted in improved resistance to biotic and abiotic stress. [See online article for color version of this figure.]

improved drought resistance. In Arabidopsis, *CBF1*, *CBF2*, and *CBF3* (also known as *DREB1b*, *DREB1c*, and *DREB1a*, respectively) play key roles in cold, salt, and drought stress responses via binding to the C-repeat/dehydration-responsive element cis-acting element of several stress-responsive genes (Novillo et al., 2012). Because *AtZAT6* positively regulates the expression of SA-dependent *PR1/PR2/PR5* and *CBF1/CBF2/CBF3* via directly binding to the TACAAT elements in the promoters, the positive regulation of pathogen-related genes and *CBF1/CBF2/CBF3* was also contributed to *AtZAT6*-mediated abiotic stress resistance.

It should be mentioned that ectopic overexpression of tagged TFs obviously does not reflect the natural situation, and it may have some limitations in dissecting the in vivo roles of the TF. However, the ChIP result (the direct binding of *AtZAT6* to the TACAAT elements of the promoters of *EDS1*, *PAD4*, *PR1*, *PR2*, *PR5*, *CBF1*, *CBF2*, and *CBF3*) exhibited high consistency with changes of gene expression levels in *AtZAT6*-overexpressing and knockdown plants relative to wild-type plants as well as the phenotypes of stress resistance. Based on the above observations, a working model is proposed in Figure 9 to depict the mechanism of *AtZAT6* involved in plant biotic and abiotic stress responses. Biotic and abiotic stresses largely induced the expression of *AtZAT6*. *AtZAT6* could bind to the TACAAT elements of the promoters of *EDS1*, *PAD4*, *PR1*, *PR2*, *PR5*, *CBF1*, *CBF2*, and *CBF3*, leading to accumulation of SA and up-regulation of stress-responsive genes. Moreover, increased expression of *AtZAT6* also resulted in higher ROS accumulation. All of these changes resulted in improved resistance to biotic and abiotic stress in *AtZAT6* overexpressors.

Taken together, this study indicates that *AtZAT6* plays important roles in plant development and positively modulates biotic and abiotic stress resistances by activating the expression levels of SA-related genes and *CBF* genes.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The Arabidopsis (*Arabidopsis thaliana*) seeds of Col-0 ecotypes were first surface sterilized with 50% (v/v) bleach and 0.1% (v/v) Triton X-100 and then thoroughly washed three times with sterile water. After stratification at 4°C for 3 d in darkness, Arabidopsis seeds were grown in soil or on MS medium containing 1% (w/v) Suc and cultured in a growth chamber. The growth chamber was controlled at an irradiance of 120 μmol quanta m^{-2} sec^{-1} at 23°C ± 2°C with 65% relative humidity under 16-h-light and 8-h-dark cycles. Nutrient solution was supplied with water every three days to keep plant growth.

Generation of *AtZAT6* Transgenic Lines

For the *ProZAT6*::*GUS* transgenic construct, the promoter regions of *AtZAT6* were amplified by PCR and inserted into SalI/BamHI sites of pBI101.2 vector with kanamycin resistance. For the overexpressing *AtZAT6* transgenic construct, the full-length coding sequence of *AtZAT6* was inserted into SmaI/XhoI sites of pBIM vector with kanamycin resistance under the control of the *Cauliflower mosaic virus* 35S promoter (Yang et al., 2005). For the *AtZAT6* and GFP coexpressing construct, the coding regions of *AtZAT6* were amplified by PCR and inserted into SmaI/XhoI sites of the *35S*::*EGFP* vector with Basta resistance (Cutler et al., 2000). For the amiR-*ZAT6* transgenic construct, amiR-ZAT6 fragments were amplified from the plasmid pRS300 by PCR using specific primers from Web MicroRNA Designer 3 (http://wmd3.weigelworld.org/cgi-bin/webapp.cgi; Schwab et al., 2006), and then the amiR-ZAT6 fragment was inserted into the SmaI site of pBARN vector with Basta resistance (LeClere and Bartel, 2001). The primers for vector constructs are listed in Supplemental Table S2. After introduction into Arabidopsis wild-type (Col-0) plants, homozygous transgenic plants were selected on MS medium containing 1% (w/v) Suc by kanamycin or Basta resistance and were confirmed by PCR analysis.

RNA Isolation and Quantitative Real-Time PCR

RNA isolation and quantitative real-time PCR was performed as described by Shi et al. (2013b). The quantitative real-time PCR was carried out with Sybr-green fluorescence using a CFX96 Real Time System (Bio-Rad). System software by the comparative ΔΔcycle threshold method was used for quantification, and the expression levels of target genes were normalized to the amount of the housekeeping gene *ubiquitin10* (*UBQ10*). At least three independent experiments were performed for real-time PCR, and three technical replicates were used in each independent experiment. The specific primers of analyzed genes for real-time PCR are listed in Supplemental Table S3.

Subcellular Localization Analysis

For subcellular localization analysis, 5-d-old seedling roots of *35S*::*GFP-ZAT6* transgenic plants were analyzed. GFP signals were detected using the Olympus FluoView 1000 confocal laser scanning microscope, and 20 μg mL^{-1} propidium iodide was used for cell wall staining in GFP lines.

GUS Staining and Quantification of GUS Activity

GUS staining and quantification of GUS activity was performed using the method described by Jefferson et al. (1987).

Determination of ROS Level

DAB staining and NBT staining were performed as previously described by Shi et al. (2013b). The concentrations of H_2O_2, $O_2\bullet^-$, GSH, and GSSG were also quantified as described by Shi et al. (2013a, 2013b).

Trypan Blue Staining

Trypan blue staining was carried out on 28-d-old plant leaves in lactophenol trypan blue solution as described by Yang et al. (2005). Then, the stained plants were transferred to 70% (v/v) ethanol to remove the chlorophyll and photographed.

Quantification of SA Content

Free and conjugated SA in 28-d-old Arabidopsis leaves were extracted and quantified by HPLC as previously described (Qiu et al., 2007) with minor modification. For HPLC assay, samples were passed over a column with a length of 15 cm and an internal diameter of 4.6 mm filled with 5 μm of reverse phase material (HeSep C18-T; Weltech) at 25°C; the solvent flow was 0.8 mL min^{-1} (methanol:water with 0.1% [w/v] H_3PO_4 = 1:1).

Plant Disease Resistance Assay

For disease resistance assay, 28-d-old plant leaves were infected as described by Shi et al. (2012) with the virulent *Pseudomonas syringae* pv *tomato* (*Pst*) DC3000 and the avirulent *Pst* DC3000(*avrRpt2*) at optical densities at 600 nm of 0.001 and 0.02, respectively. Inoculation with 10 mM $MgCl_2$ was used as mock treatment. At 0 and 3 dpi, leaf discs within the infiltrated area were taken immediately, and the bacterial growth from infected Arabidopsis leaves and symptoms were monitored as described (Shi et al., 2012). For SAR assay,

local leaves were first infected with *Pst* DC3000(*avrRpt2*) for 1 d. Then, the distal, uninfected plant leaves were infected with *Pst* DC3000, and the growth of *Pst* DC3000 on distal plant leaves was assayed at 0 and 3 dpi of bacterial infection. At least three independent experiments were performed, and 20 independent leaf discs were harvest in each independent experiment.

Plant Abiotic Stress Resistance Assay

Plant abiotic stress resistance was assayed as described by Shi et al. (2013b), and survival rate was determined 7 d after recovery from the abiotic stress treatments.

Transient Expression Assay in Tobacco Leaves

To confirm the interaction between AtZAT6 and the TACAAT element, the transient expression assay in tobacco (*Nicotiana tabacum*) leaves were performed as described by Huang et al. (2013). The m35S-GUS, TACAAT-m35S-GUS, and TAAAAT-m35S-GUS were constructed by inserting PCR products of mini35S, 4×TACAAT-mini35S, and 4×TAAAAT-mini35S from the minimal-100 *Cauliflower mosaic virus* 35S-pCAMBIA1391 plastid (Huang et al., 2013) into Hin*dIII* and Bam*HI* sites of the pCAMBIA1391Z vector. The corresponding primers for vector construction are listed in Supplemental Table S2. For the transient expression analysis, the pBIM vector and AtZAT6-pBIM construct were used as the effectors, and the m35S-GUS (mini35S-pCAMBIA1391Z), TACAAT-m35S-GUS (4×TACAAT-mini35S-pCAMBIA1391Z), and TAAAAT-m35S-GUS (4×TAAAAT-mini35S-pCAMBIA1391Z) constructs were used as the reporters. The effector and reporter were transformed into *Agrobacterium tumefaciens* strain GV3101 and coinfected into tobacco leaves as described by Huang et al. (2013), and the 35S::GFP plastid (Cutler et al., 2000) was coinjected as an internal standard in each infection. The GUS activity and GFP fluorescence intensity were quantified using the Infinite M200 Microplate reader (Tecan).

ChIP Assay

ChIP was performed as described by Bowler et al. (2004). Briefly, 2 g of 14-d-old *35S::GFP-ZAT6* transgenic plants were harvested and then immersed in 1% (v/v) formaldehyde for cross linking the DNA with DNA-binding proteins. Next, the chromatin pellets were extracted and sheared by sonication as described by Bowler et al. (2004), and the anti-GFP antibody (AG281; Beyotime) was used to immunoprecipitate the DNA-ZAT6 complexes. The DNA was released with proteinase K, and the enrichment of DNA fragments was determined using quantitative real-time PCR with the specific primers listed in Supplemental Table S3.

Statistical Analysis

All experiments in this study were repeated at least three times, and every experiment was harvested from at least 15 seedlings per genotype. Student's *t* test was used to determine the significant difference between wild-type and transgenic lines, and asterisks indicate the significant difference of $P < 0.05$ compared with the wild type.

Nucleotide sequence data for the genes described in this article are available from The Arabidopsis Information Resource under the following accession numbers: *ZAT6* (At5g04340), *UBQ10* (At4g05320), *ACTIN2* (At3g18780), *EDS1* (At3g48090), *PAD4* (At3g52430), *PR1* (At2g14610), *PR2* (At3g57260), *PR3* (At3g12500), *PR4* (At3g04720), *PR5* (At1g75040), *CBF1* (At4g25490), *CBF2* (At4g25470), *CBF3* (At4g25480), *CBF4* (At5g51990), *DREB2A* (At5g05410), and *DREB2B* (At3g11020).

Supplemental Data

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

Supplemental Figure S1. Expression level changes of *AtZAT6* under abiotic and biotic stress conditions.

Supplemental Figure S2. Transactivation activity of AtZAT6 in yeast.

Supplemental Figure S3. Phenotypes of 21-d-old wild-type and *AtZAT6*-overexpressing T1 generation plants.

Supplemental Figure S4. The expression level of *AtZAT6* in *AtZAT6* knockdown plants.

Supplemental Figure S5. Phenotypes of 7-d-old wild-type and *AtZAT6*-overexpressing plants on MS plate.

Supplemental Figure S6. Modulation of AtZAT6 expression affects glutathione pool and redox state.

Supplemental Figure S7. Phenotype of *35S::GFP-ZAT6* transgenic plants.

Supplemental Table S1. Modulation of morphological phenotypes by *AtZAT6* expression.

Supplemental Table S2. Primers used for vector construction.

Supplemental Table S3. Primers used for quantitative real-time PCR.

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LITERATURE CITED

Bent AF, Mackey D (2007) Elicitors, effectors, and R genes: the new paradigm and a lifetime supply of questions. Annu Rev Phytopathol **45:** 399–436

Bi D, Cheng YT, Li X, Zhang Y (2010) Activation of plant immune responses by a gain-of-function mutation in an atypical receptor-like kinase. Plant Physiol **153:** 1771–1779

Bogamuwa S, Jang JC (2013) The *Arabidopsis* tandem CCCH zinc finger proteins AtTZF4, 5 and 6 are involved in light-, abscisic acid- and gibberellic acid-mediated regulation of seed germination. Plant Cell Environ **36:** 1507–1519

Bowler C, Benvenuto G, Laflamme P, Molino D, Probst AV, Tariq M, Paszkowski J (2004) Chromatin techniques for plant cells. Plant J **39:** 776–789

Cheng YT, Li Y, Huang S, Huang Y, Dong X, Zhang Y, Li X (2011) Stability of plant immune-receptor resistance proteins is controlled by SKP1-CULLIN1-F-box (SCF)-mediated protein degradation. Proc Natl Acad Sci USA **108:** 14694–14699

Ciftci-Yilmaz S, Morsy MR, Song L, Coutu A, Krizek BA, Lewis MW, Warren D, Cushman J, Connolly EL, Mittler R (2007) The EAR-motif of the Cys2/His2-type zinc finger protein Zat7 plays a key role in the defense response of Arabidopsis to salinity stress. J Biol Chem **282:** 9260–9268

Cutler SR, Ehrhardt DW, Griffitts JS, Somerville CR (2000) Random GFP: cDNA fusions enable visualization of subcellular structures in cells of Arabidopsis at a high frequency. Proc Natl Acad Sci USA **97:** 3718–3723

Davletova S, Schlauch K, Coutu J, Mittler R (2005) The zinc-finger protein Zat12 plays a central role in reactive oxygen and abiotic stress signaling in Arabidopsis. Plant Physiol **139:** 847–856

Devaiah BN, Nagarajan VK, Raghothama KG (2007) Phosphate homeostasis and root development in Arabidopsis are synchronized by the zinc finger transcription factor ZAT6. Plant Physiol **145:** 147–159

Gimenez-Ibanez S, Solano R (2013) Nuclear jasmonate and salicylate signaling and crosstalk in defense against pathogens. Front Plant Sci **4:** 72

Huang XS, Wang W, Zhang Q, Liu JH (2013) A basic helix-loop-helix transcription factor, *PtrbHLH*, of *Poncirus trifoliata* confers cold tolerance and modulates peroxidase-mediated scavenging of hydrogen peroxide. Plant Physiol **162:** 1178–1194

Jefferson RA, Kavanagh TA, Bevan MW (1987) GUS fusions: β-glucuronidase as a sensitive and versatile gene fusion marker in higher plants. EMBO J **6:** 3901–3907

Kazan K, Manners JM (2009) Linking development to defense: auxin in plant-pathogen interactions. Trends Plant Sci **14:** 373–382

Kieffer M, Neve J, Kepinski S (2010) Defining auxin response contexts in plant development. Curr Opin Plant Biol **13:** 12–20

Kodaira KS, Qin F, Tran LSP, Maruyama K, Kidokoro S, Fujita Y, Shinozaki K, Yamaguchi-Shinozaki K (2011) Arabidopsis Cys2/His2

zinc-finger proteins AZF1 and AZF2 negatively regulate abscisic acid-repressive and auxin-inducible genes under abiotic stress conditions. Plant Physiol **157:** 742–756

Kong Q, Qu N, Gao M, Zhang Z, Ding X, Yang F, Li Y, Dong OX, Chen S, Li X, et al (2012) The MEKK1-MKK1/MKK2-MPK4 kinase cascade negatively regulates immunity mediated by a mitogen-activated protein kinase kinase kinase in *Arabidopsis*. Plant Cell **24:** 2225–2236

Kunkel BN, Bent AF, Dahlbeck D, Innes RW, Staskawicz BJ (1993) *RPS2*, an *Arabidopsis* disease resistance locus specifying recognition of *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. Plant Cell **5:** 865–875

Lamb C, Dixon RA (1997) The oxidative burst in plant disease resistance. Annu Rev Plant Physiol Plant Mol Biol **48:** 251–275

LeClere S, Bartel B (2001) A library of Arabidopsis 35S-cDNA lines for identifying novel mutants. Plant Mol Biol **46:** 695–703

Li Y, Li S, Bi D, Cheng YT, Li X, Zhang Y (2010a) SRFR1 negatively regulates plant NB-LRR resistance protein accumulation to prevent autoimmunity. PLoS Pathog **6:** e1001111

Li Y, Tessaro MJ, Li X, Zhang Y (2010b) Regulation of the expression of plant *resistance* gene *SNC1* by a protein with a conserved BAT2 domain. Plant Physiol **153:** 1425–1434

Liu J, Ding P, Sun T, Nitta Y, Dong O, Huang X, Yang W, Li X, Botella JR, Zhang Y (2013a) Heterotrimeric G proteins serve as a converging point in plant defense signaling activated by multiple receptor-like kinases. Plant Physiol **161:** 2146–2158

Liu WX, Zhang FC, Zhang WZ, Song LF, Wu WH, Chen YF (2013b) Arabidopsis Di19 functions as a transcription factor and modulates *PR1*, *PR2*, and *PR5* expression in response to drought stress. Mol Plant **6:** 1487–1502

Liu X, Zhang H, Zhao Y, Feng Z, Li Q, Yang HQ, Luan S, Li J, He ZH (2013c) Auxin controls seed dormancy through stimulation of abscisic acid signaling by inducing ARF-mediated ABI3 activation in Arabidopsis. Proc Natl Acad Sci USA **110:** 15485–15490

Liu XM, Nguyen XC, Kim KE, Han HJ, Yoo J, Lee K, Kim MC, Yun DJ, Chung WS (2013d) Phosphorylation of the zinc finger transcriptional regulator ZAT6 by MPK6 regulates Arabidopsis seed germination under salt and osmotic stress. Biochem Biophys Res Commun **430:** 1054–1059

Miller G, Shulaev V, Mittler R (2008) Reactive oxygen signaling and abiotic stress. Physiol Plant **133:** 481–489

Mito T, Seki M, Shinozaki K, Ohme-Takagi M, Matsui K (2011) Generation of chimeric repressors that confer salt tolerance in Arabidopsis and rice. Plant Biotechnol J **9:** 736–746

Mittler R, Kim Y, Song L, Coutu J, Coutu A, Ciftci-Yilmaz S, Lee H, Stevenson B, Zhu JK (2006) Gain- and loss-of-function mutations in *Zat10* enhance the tolerance of plants to abiotic stress. FEBS Lett **580:** 6537–6542

Nguyen XC, Kim SH, Lee K, Kim KE, Liu XM, Han HJ, Hoang MHT, Lee SW, Hong JC, Moon YH, et al (2012) Identification of a C_2H_2-type zinc finger transcription factor (ZAT10) from Arabidopsis as a substrate of MAP kinase. Plant Cell Rep **31:** 737–745

Novillo F, Medina J, Rodríguez-Franco M, Neuhaus G, Salinas J (2012) Genetic analysis reveals a complex regulatory network modulating *CBF* gene expression and Arabidopsis response to abiotic stress. J Exp Bot **63:** 293–304

Pieterse CM, Van der Does D, Zamioudis C, Leon-Reyes A, Van Wees SC (2012) Hormonal modulation of plant immunity. Annu Rev Cell Dev Biol **28:** 489–521

Qiu D, Xiao J, Ding X, Xiong M, Cai M, Cao Y, Li X, Xu C, Wang S (2007) *OsWRKY13* mediates rice disease resistance by regulating defense-related genes in salicylate- and jasmonate-dependent signaling. Mol Plant Microbe Interact **20:** 492–499

Qiu JL, Zhou L, Yun BW, Nielsen HB, Fiil BK, Petersen K, Mackinlay J, Loake GJ, Mundy J, Morris PC (2008) Arabidopsis mitogen-activated protein kinase kinases MKK1 and MKK2 have overlapping functions in defense signaling mediated by MEKK1, MPK4, and MKS1. Plant Physiol **148:** 212–222

Rizhsky L, Davletova S, Liang H, Mittler R (2004) The zinc finger protein Zat12 is required for cytosolic ascorbate peroxidase 1 expression during oxidative stress in Arabidopsis. J Biol Chem **279:** 11736–11743

Schwab R, Ossowski S, Riester M, Warthmann N, Weigel D (2006) Highly specific gene silencing by artificial microRNAs in *Arabidopsis*. Plant Cell **18:** 1121–1133

Seo PJ, Park CM (2010) *MYB96*-mediated abscisic acid signals induce pathogen resistance response by promoting salicylic acid biosynthesis in Arabidopsis. New Phytol **186:** 471–483

Shi H, Ye T, Chan Z (2013a) Comparative proteomic and physiological analyses reveal the protective effect of exogenous polyamines in the bermudagrass (*Cynodon dactylon*) response to salt and drought stresses. J Proteome Res **12:** 4951–4964

Shi H, Ye T, Chen F, Cheng Z, Wang Y, Yang P, Zhang Y, Chan Z (2013b) Manipulation of arginase expression modulates abiotic stress tolerance in Arabidopsis: effect on arginine metabolism and ROS accumulation. J Exp Bot **64:** 1367–1379

Shi H, Ye T, Zhong B, Liu X, Jin R, Chan Z (April 16, 2014a) AtHAP5A modulates freezing stress resistance in Arabidopsis through binding to CCAAT motif of *AtXTH21*. New Phytol http://dx.doi.org/10.1111/nph.12812

Shi H, Ye T, Zhu JK, Chan Z (May 27, 2014b) Constitutive production of nitric oxide leads to enhanced drought stress resistance and extensive transcriptional reprogramming in Arabidopsis. J Exp Bot http://dx.doi.org/10.1093/jxb/eru184

Shi HT, Li RJ, Cai W, Liu W, Wang CL, Lu YT (2012) Increasing nitric oxide content in *Arabidopsis thaliana* by expressing rat neuronal nitric oxide synthase resulted in enhanced stress tolerance. Plant Cell Physiol **53:** 344–357

Winter D, Vinegar B, Nahal H, Ammar R, Wilson GV, Provart NJ (2007) An "Electronic Fluorescent Pictograph" browser for exploring and analyzing large-scale biological data sets. PLoS ONE **2:** e718

Xu S, Zhang Z, Jing B, Gannon P, Ding J, Xu F, Li X, Zhang Y (2011) Transportin-SR is required for proper splicing of *resistance* genes and plant immunity. PLoS Genet **7:** e1002159

Yang DL, Yao J, Mei CS, Tong XH, Zeng LJ, Li Q, Xiao LT, Sun TP, Li J, Deng XW, et al (2012a) Plant hormone jasmonate prioritizes defense over growth by interfering with gibberellin signaling cascade. Proc Natl Acad Sci USA **109:** E1192–E1200

Yang LX, Wang RY, Ren F, Liu J, Cheng J, Lu YT (2005) *AtGLB1* enhances the tolerance of Arabidopsis to hydrogen peroxide stress. Plant Cell Physiol **46:** 1309–1316

Yang Y, Zhang Y, Ding P, Johnson K, Li X, Zhang Y (2012b) The ankyrin-repeat transmembrane protein BDA1 functions downstream of the receptor-like protein SNC2 to regulate plant immunity. Plant Physiol **159:** 1857–1865

Yu H, Chen X, Hong YY, Wang Y, Xu P, Ke SD, Liu HY, Zhu JK, Oliver DJ, Xiang CB (2008) Activated expression of an *Arabidopsis* HD-START protein confers drought tolerance with improved root system and reduced stomatal density. Plant Cell **20:** 1134–1151

Zhang Y, Xu S, Ding P, Wang D, Cheng YT, He J, Gao M, Xu F, Li Y, Zhu Z, et al (2010a) Control of salicylic acid synthesis and systemic acquired resistance by two members of a plant-specific family of transcription factors. Proc Natl Acad Sci USA **107:** 18220–18225

Zhang Y, Yang Y, Fang B, Gannon P, Ding P, Li X, Zhang Y (2010b) *Arabidopsis snc2-1D* activates receptor-like protein-mediated immunity transduced through WRKY70. Plant Cell **22:** 3153–3163

Zhang Z, Li Q, Li Z, Staswick PE, Wang M, Zhu Y, He Z (2007) Dual regulation role of *GH3.5* in salicylic acid and auxin signaling during Arabidopsis-*Pseudomonas syringae* interaction. Plant Physiol **145:** 450–464

Zhang Z, Wu Y, Gao M, Zhang J, Kong Q, Liu Y, Ba H, Zhou J, Zhang Y (2012) Disruption of PAMP-induced MAP kinase cascade by a *Pseudomonas syringae* effector activates plant immunity mediated by the NB-LRR protein SUMM2. Cell Host Microbe **11:** 253–263

Zhou Z, Sun L, Zhao Y, An L, Yan A, Meng X, Gan Y (2013) *Zinc Finger Protein 6* (*ZFP6*) regulates trichome initiation by integrating gibberellin and cytokinin signaling in *Arabidopsis thaliana*. New Phytol **198:** 699–708

Zhu Z, Xu F, Zhang Y, Cheng YT, Wiermer M, Li X, Zhang Y (2010) Arabidopsis resistance protein SNC1 activates immune responses through association with a transcriptional corepressor. Proc Natl Acad Sci USA **107:** 13960–13965