

# Overexpression of AtMYB52 Confers ABA Hypersensitivity and Drought Tolerance

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**We carried out activation tagging screen to isolate genes regulating abscisic acid (ABA) response. From the screen of approximately 10,000 plants, we isolated ca 100 ABA response mutants. We characterized one of the mutants, designated *ahs1*, in this study. The mutant is ABA-hypersensitive, and *AtMYB52* was found to be activated in the mutant. Overexpression analysis to recapitulate the mutant phenotypes demonstrated that *AtMYB52* confers ABA-hypersensitivity during postgermination growth. Additionally, *AtMYB52* overexpression lines were drought-tolerant and their seedlings were salt-sensitive. Changes in the expression levels of a few genes involved in ABA response or cell wall biosynthesis were also observed. Together, our data suggest that *AtMYB52* is involved in ABA response. Others previously demonstrated that *AtMYB52* regulates cell wall biosynthesis; thus, our results imply a possible connection between ABA response and cell wall biosynthesis.**

## INTRODUCTION

The plant hormone abscisic acid (ABA) affects plant growth and development throughout their life cycle from seed germination to seed maturation (Finkelstein, 2002). Although high concentrations of exogenous ABA inhibit both seed germination and postgermination growth, ABA is necessary for normal plant growth, and ABA-deficient mutants grow poorly under normal growth conditions. During vegetative growth, the endogenous ABA level increases under unfavorable environmental conditions, thereby helping plants cope with the adverse conditions (Xiong et al., 2002). In particular, ABA plays an essential role in adaptive responses to water deficit conditions by controlling stomatal movement to prevent water loss through transpiration (Schroeder et al., 2001). ABA also regulates the expression of numerous stress-responsive genes that are involved in protective response (Shinozaki and Yamaguchi-Shinozaki, 2007). During seed development, ABA regulates the accumulation of storage components and prevents the embryo from precocious germination.

Numerous proteins involved in various aspects of ABA response have been identified to date (Cutler et al., 2010). These include transcription factors, kinases, phosphatases and other

signaling intermediates. Recently, several types of ABA receptors were also reported (Ma et al., 2009; Pandey et al., 2009; Park et al., 2009; Shen et al., 2006), and a core of the ABA signaling network was delineated. The ABA signaling pathway has been reconstituted *in vitro* using several key components that include the ABA receptor PYR1, the type 2C protein phosphatase ABI1, the protein kinase SnRK2.6 and the bZIP class transcription factor ABF2/AREB1 (Fujii et al., 2009).

A variety of transcription factors are involved in the regulation of ABA-responsive gene expression (Yamaguchi-Shinozaki and Shinozaki, 2005). A small subfamily of bZIP proteins named ABFs/AREBs regulates ABA-responsive genes via the G box-type ABA response element (ABRE) (i.e. PyACGTGGC), which is present in numerous ABA-regulated genes (Kim, 2006). CBF/DREB and other AP2 domain proteins are known as positive or negative regulators of ABA and/or abiotic stress responses. MYB proteins such as AtMYB2, AtMYB96, AtMYB15, and AtMYB44 also regulate ABA and abiotic stress responses. Additionally, HD-ZIP, NAC, WRKY, or ZFHD proteins are known to mediate ABA and/or stress responses (Berri et al., 2009; Yamaguchi-Shinozaki and Shinozaki, 2005).

MYB proteins are involved in ABA and stress responses as well as many other cellular processes (Yanhui et al., 2006). A series of recent studies showed that a number of MYB genes, including MYB52, are involved in the regulation of secondary cell wall biosynthesis. For instance, MYB58 and MYB63 are regulators of lignin biosynthesis (Zhou et al., 2009), and MYB103, MYB85, MYB52 and MYB54 control secondary wall thickening (Zhong et al., 2008). Among the MYB transcription factors, hierarchical relationships exist, and it has been demonstrated that MYB52 is a downstream target of MY46, which is a master switch for secondary cell wall formation in Arabidopsis (Ko et al., 2009).

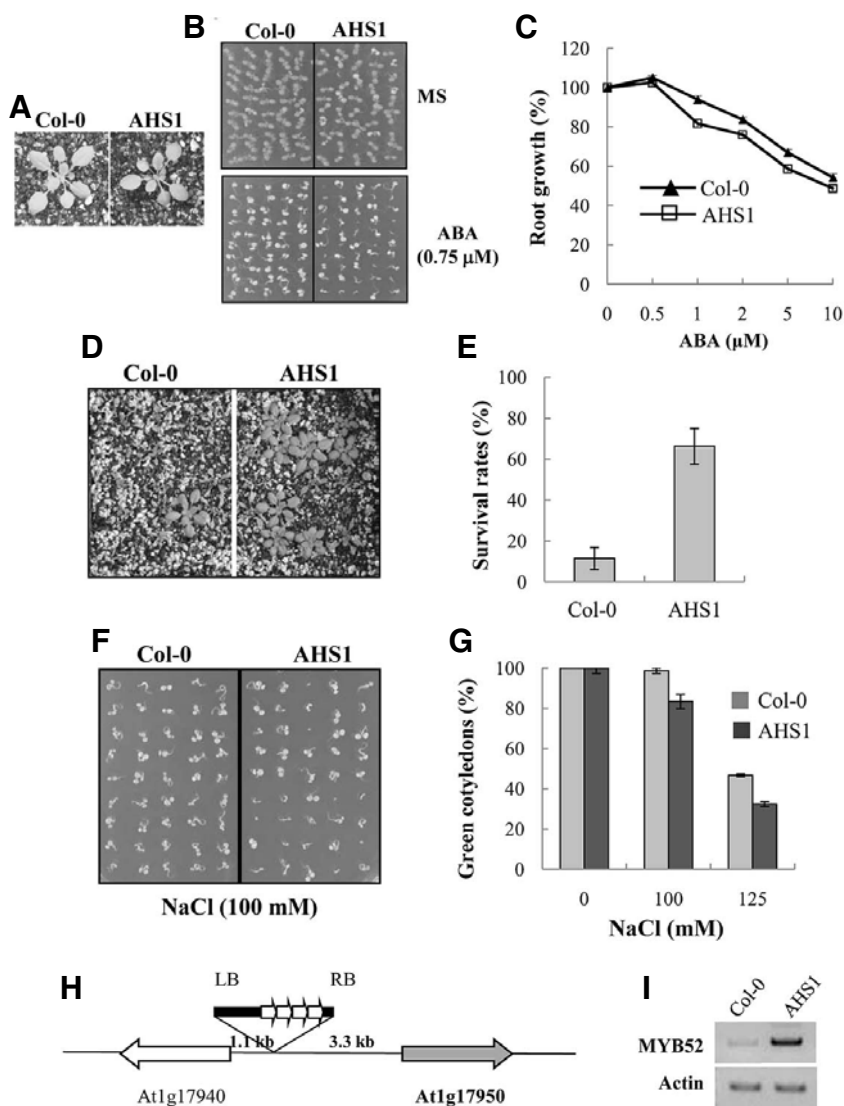
In the present study, we isolated an ABA response mutant by activation tagging screen. The mutant, referred to as *ahs1*, is hypersensitive to ABA, and determination of T-DNA insertion site and subsequent expression analysis revealed that *MYB52* was activated in the mutant. Recapitulation experiments to confirm its role in ABA response showed that MYB52 is involved in ABA and stress responses. Taken together, the results presented herein suggest a possible connection between ABA response and cell wall biosynthesis.

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**Fig. 1.** The phenotypes of the tagging mutant *ahs1*. (A) Growth of *ahs1* in soil. Plants were grown in soil for three weeks. (B, C) ABA sensitivity of *ahs1*. Seeds were germinated and grown in a medium containing 0.75 μM ABA for seven days (B). For root elongation assay in (C), seeds were germinated and grown in an ABA-free medium for three days, the seedlings were transferred to media containing various concentrations of ABA, and primary root growth was measured five days after the transfer. Experiments were carried out in triplicates ( $n = 6$ ), and the small bars indicate the standard errors. (D, E) Drought tolerance of *ahs1*. Water was withheld from 11 day-old seedlings for 11 days, after which they were re-watered. The picture in (D) was taken two days after re-watering. The survival rates in (E) represent the means of three independent experiments ( $n = 20$  each), and the small bars indicate the standard errors. (F, G) Salt tolerance of *ahs1*. Seeds were germinated and grown in media containing 100 mM or 150 mM NaCl for four days, and seedlings with green cotyledons were then counted. All experiments were conducted in triplicates ( $n = 50$ ), and standard errors are indicated by the small bars. (H) A diagram showing the position of T-DNA insertion in the AHS mutant. (I) The expression level of *AtMYB52* (*At1g17950*) (*MYB52*) was determined by RT-PCR. *Actin-1* was used as an internal control.

## MATERIALS AND METHODS

### Plant growth and generation of activation-tagged lines

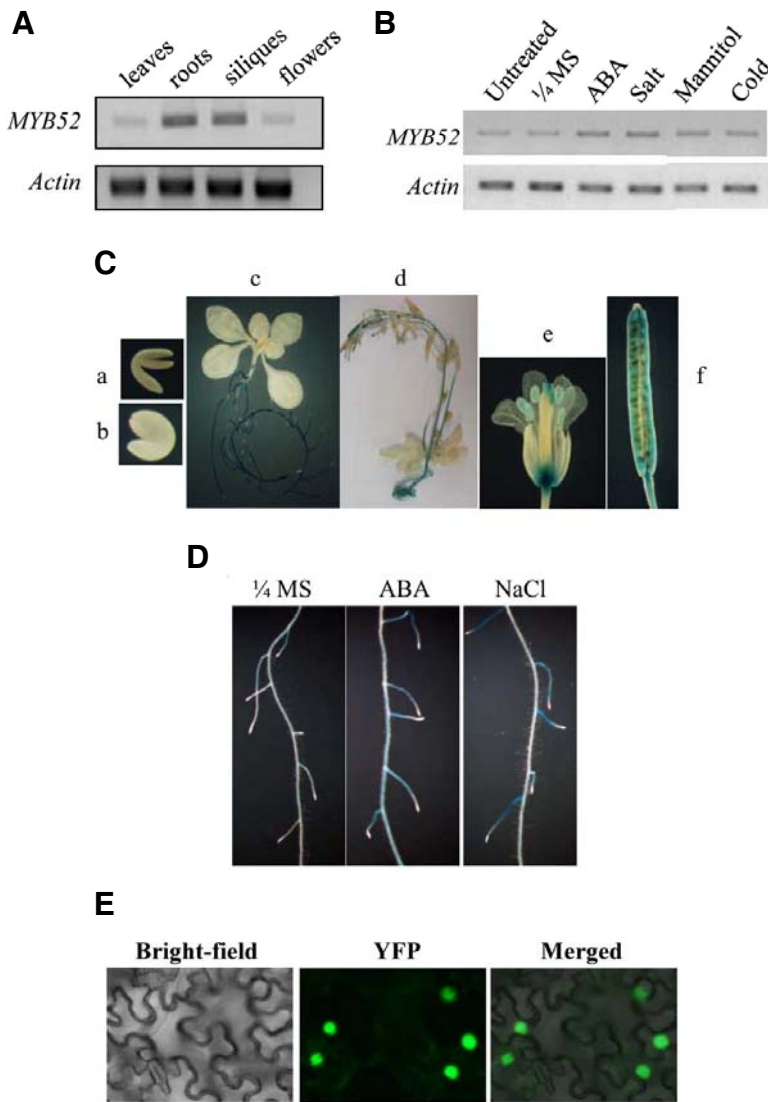
*Arabidopsis thaliana* ecotype Columbia (Col-0) and Landsberg *erecta* (*Ler*) were used in this study. The plants were grown under long day condition (16-h-light/8-h-dark cycle) at 22°C aseptically or on soil. For aseptic growth, seeds were surface-sterilized by treatment with 70% ethanol for 1 min and with 30% bleach for 5 min, after which they were washed with sterile water five times before planting. For soil growth, seeds were sown on a mixture of vermiculite, perlite and peat moss (1:1:1 by weight), irrigated with 0.1% Hyponex (Hyponex Co., USA), placed at 4°C for 3-5 days in the dark to break residual dormancy, and then transferred to normal growth conditions. Plants were watered once a week.

To generate activation-tagged transgenic plants, *Arabidopsis* plants (Col-0) were transformed with *A. tumefaciens* strain GV3101 harboring the vector pSKI015 (Weigel et al., 2000) according to Bechtold and Pelletier (Bechtold and Pelletier, 1998). Approximately 25,000 basta-resistant plants were recovered, and seeds were collected in pools of ca 100 transgenic plants. To screen for ABA-hypersensitive mutants, seeds

from each pool were plated and germinated in the medium containing 0.3 μM ABA, and seedlings exhibiting abnormal germination and/or postgermination growth were selected and transferred to ABA-free medium. The plants were subsequently transferred to soil and their seeds were harvested. A total of ca 100 mutants were isolated from the primary screen of 100 pools, which is equivalent to 10,000 transgenic plants. The seeds from individual plants were then germinated and their phenotypes were confirmed. For analysis of the *ahs1* mutant phenotypes shown in Fig. 1, we employed one of the heterozygous sublines (#8-3) because we could not recover homozygous lines. The T-DNA insertion site in the *ahs1* mutant was determined by sequencing the left border flanking sequence after rescuing the plasmid, which was obtained by ligation of the *ahs1* genomic DNA digested with *Spe* I according to Weigel et al. (2000).

### Generation of transgenic lines and phenotype analysis

To generate the *AHS1* overexpression (OX) lines, the coding region of *AtMYB52* was amplified using the primer set 5'-TGC TCT AGA GTA TTA AAA AAT GAT GTG TAG TCG A-3' and 5'-GAC AAA TTA ACA TAA ACC CTG AGA G-3'. After *Xba*I



**Fig. 2.** Expression patterns of AtMYB52. (A) The expression of AtMYB52 (MYB52) in various tissues was determined by RT-PCR. (B) Induction patterns of AtMYB52 were determined by RT-PCR. ABA (100  $\mu$ M), salt (150 mM NaCl) and mannitol (600 mM) treatments were conducted for 4 h. For cold treatment, plants were placed at 4 C for 24 h before RNA isolation. Untreated, control plants without any treatments. (C) GUS staining pattern of transgenic plants harboring the AtMYB52 promoter-GUS reporter gene construct. a, immature embryo; b, mature embryo; c, 11 day-old seedling; d, five week-old whole plant; e, flower; f, silique. GUS staining was for 24 h. (D) GUS induction patterns. Plants were treated with 100  $\mu$ M ABA (ABA) or 250 mM NaCl (NaCl) before GUS staining. GUS staining was conducted for 6 h. (E) Sub-cellular localization of AtMYB52 was investigated by Agroinfiltration of tobacco leaves using an AtMYB52-YFP fusion construct. Tobacco leaves were observed with a fluorescence microscope 40 h after infiltration.

digestion, the coding region was cloned into pBI121 (Jefferson, 1987), which was prepared by removing the GUS coding after the *Xba*I-*Eco*CR1 digestion. To prepare the AtMYB52 promoter-GUS reporter construct, 2.4 kb of the 5' flanking sequence was amplified employing the primer set 5'-TAG AAG CTT GTG GTT TGA TG G TAT TGA TTA AGT T-3' and 5'-TTT TTA ATA CCT CTC TCC TTT TGA TC-3' and then cloned into the *Hind*III-*Sma*I sites of pBI101.2 (Jefferson, 1987) after *Hind*III digestion. The constructs were introduced into *A. tumefaciens* strain GV3101, and Arabidopsis plants (Col-0 for the promoter-GUS lines and Ler for the OX lines) were transformed according to the method described by Bechtold and Pelletier (1998). For the analysis of OX lines, we recovered seven T3 generation homozygous lines, and the T4 generation seeds from these lines were used for phenotype analysis.

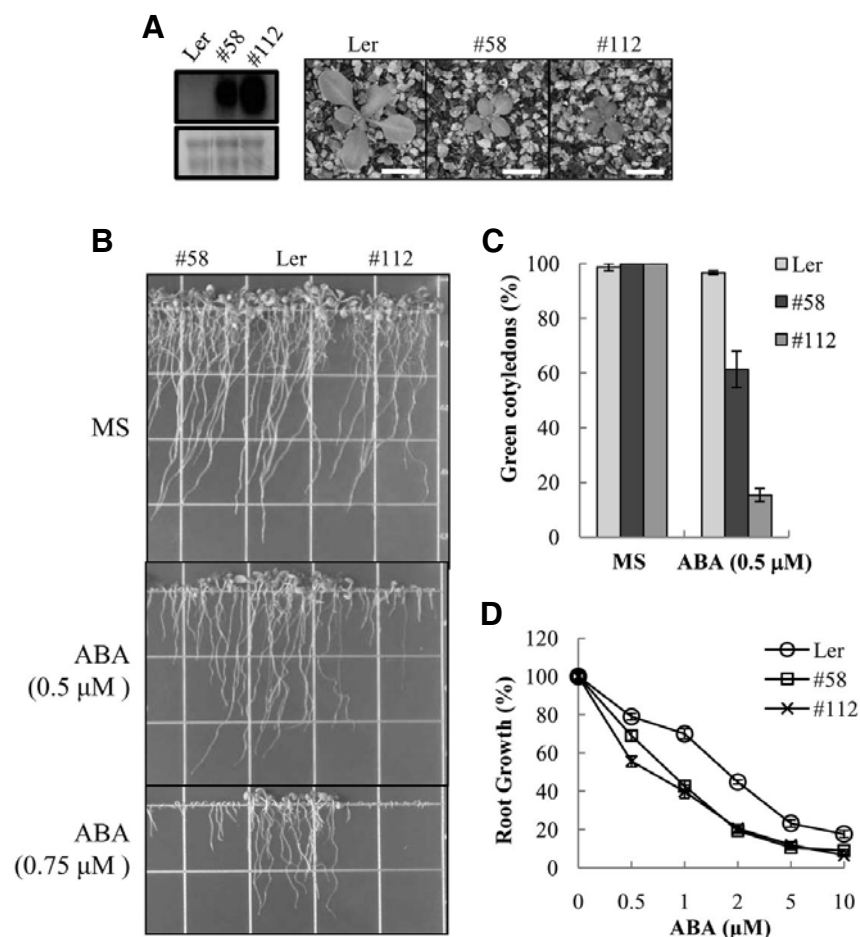
Phenotype analysis of transgenic plants was conducted as described before (Kang et al., 2002; Kim et al., 2004). For aseptic growth, seeds were treated as described above and plated on MS medium (Murashige and Skoog, 1962) solidified with 0.8% Phytoagar after cold treatment at 4°C for 3-5 days. The MS medium was supplemented with 1% sucrose, and various

concentrations of ABA or NaCl was supplemented as indicated for ABA and salt sensitivity tests. For the drought test, water was withheld from ten to eleven day-old soil-grown plants until they lost turgor completely, at which time they were re-watered and their survival rates were determined. The same number of wild type and transgenic plants were grown on the same tray to minimize experimental variations.

Histochemical GUS assay was conducted as described by Jefferson et al. (1987). Briefly, T3 homozygous plants were immersed in a staining solution containing 1 mM X-gluc (5-bromo-4-chloro-3-indolyl- $\beta$ -glucuronic acid) in the buffer (100 mM sodium phosphate, pH 7.0, 0.1 mM EDTA, 0.5 mM ferri-cyanide, 0.5 mM ferrocyanide, and 0.1% Triton X-100). Staining was conducted at 37°C for the indicated times. At the end of staining, chlorophyll was removed from the plant tissues by immersing them in 95% ethanol.

#### RNA isolation and expression analysis

RNA was isolated employing a Qiagen RNeasy plant mini kit. Northern blot analysis was performed as described previously (Kang et al., 2002). For RT-PCR analysis, possible contaminat-



**Fig. 3.** ABA sensitivity of *AtMYB52* overexpression lines. (A) Growth of the transgenic plants in soil. Plants were grown for three weeks. The expression levels of *AtMYB52* determined by Northern analysis are shown in the left panel. Ler, wild type plants. #58 and #112 denote two transgenic lines. (B) Plants were grown in media containing ABA. (C) Cotyledon greening efficiency was determined seven days after seed sowing. Experiments were conducted in triplicate ( $n = 50$  each) and the small bars represent the standard errors. (D) Root elongation assay. Seedlings were germinated and grown in a medium lacking ABA for three days, after which the seedlings were transferred to a medium containing various concentrations of ABA. Root elongation was then measured after five days. All experiments were conducted in triplicates ( $n = 6$  each) and the small bars represent the standard errors.

ing DNA was removed from RNA samples by DNase I treatment. The first strand cDNA was synthesized using Superscript III (Invitrogen) according to the supplier's instructions. For semi-quantitative RT-PCR, cDNA amplification was carried out within a linear range using gene-specific primers. For Real-Time RT-PCR, the cDNA amplification was conducted using SsoFast EvaGreen supermix in conjunction with a Bio-Rad CFX96 Real-Time PCR System (Bio-Rad). Quantitation was conducted using the CFX96 Real-Time PCR Systems software. *Actin-1* was employed as a reference gene. Primer sequences are available upon request.

#### Determination of subcellular localization of *AtMYB52*

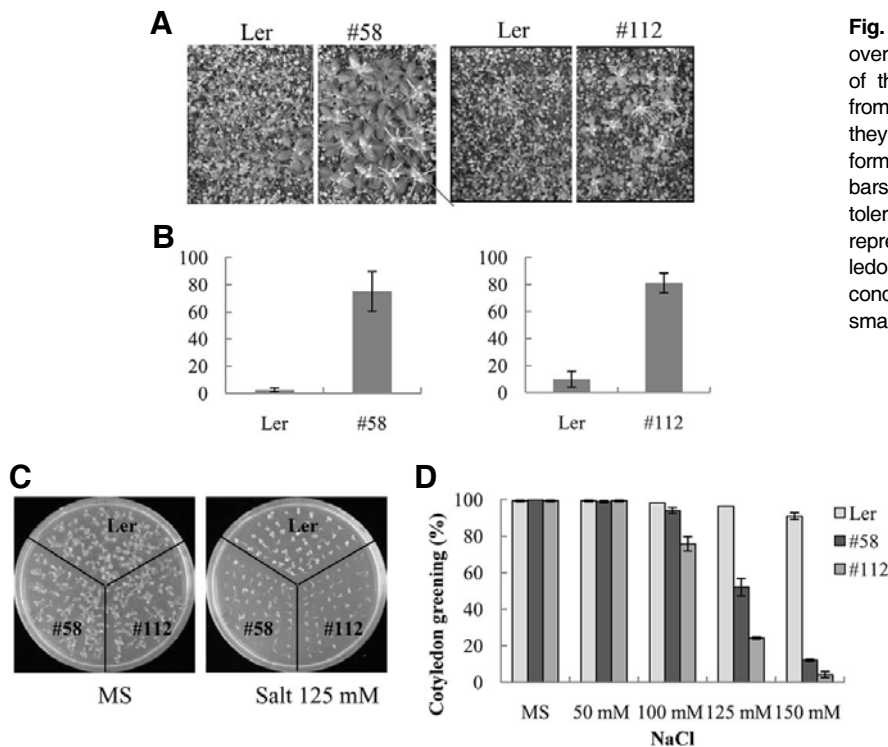
The coding region of *AtMYB52* was amplified using the primers 5'-CGG AGC TCA TGA TGT GTA GTC GAG GCC ATT G-3' and 5'-ACA TAA ACC CTG AGA GGC AGA GTT-3' and then digested with *SacI*. The amplified fragment was subsequently cloned into the *SacI-SmaI* sites of p35S-FAST/EYFP in frame with the EYFP coding region. Tobacco (*Nicotiana benthamiana*) leaves were co-infiltrated with *Agrobacterium strains* (C58C1) containing the fusion construct and p19, respectively, according to the method described by Voinnet et al. (Voinnet et al., 2003). The tobacco epidermal cells were observed with a fluorescence microscope (Olympus BX51) 40 h after infiltration.

## RESULTS

### Activation tagging screen

We performed activation tagging screen to isolate genes involved in ABA response. A library of activation-tagged transgenic plants was generated using the vector pSKI015 (Weigel et al., 2000), and mutants exhibiting altered ABA response were isolated. One of the mutants, which is designated *ahs1* (ABA-hypersensitive1), is shown in Fig. 1A. Under the normal growth condition, *ahs1* did not exhibit distinct phenotypes other than smaller plant size. However, seedling establishment (i.e., cotyledon greening) of the mutant plants was inhibited more severely by ABA than wild type plants (Fig. 1B), suggesting that it may be ABA-hypersensitive. Enhanced ABA sensitivity was also observed in the later growth stage, i.e., inhibition of primary root elongation by ABA was more pronounced in *ahs1* mutant than in wild type plants (Fig. 1C). Additionally, the mutant seedlings displayed higher survival rates under water-stress condition (Figs. 1D and 1E) and were hypersensitive to salt (Figs. 1F and 1G). Thus, our analysis of *ahs1* mutant phenotypes indicated that it is ABA-hypersensitive and that it exhibits altered drought and salt tolerance.

To determine the T-DNA insertion site in the *ahs1* mutant, the sequence flanking the left border was recovered by plasmid rescue (Weigel et al., 2000). Sequencing of the recovered genomic DNA fragment revealed that T-DNA was inserted in the intergenic region between the two genes, At1g17940 and At1g17950, which encode an unknown gene and a MYB gene,



**Fig. 4.** Drought and salt tolerance of *AtMYB52* overexpression lines. (A, B) Drought tolerance of the transgenic plants. Water was withheld from ten day-old plants for 16 days, after which they were re-watered. Experiments were performed in duplicates ( $n = 20$  each) and the small bars represent the standard errors. (C, D) Salt tolerance of the transgenic plants. (C) shows representative plants, whereas (D) shows cotyledon greening efficiency. Experiments were conducted in triplicates ( $n = 55$  each), and the small bars in (D) indicate the standard errors.

*AtMYB52*, respectively (Fig. 1H). Subsequently, RT-PCR was conducted to measure changes in the transcript levels of the two genes. As shown in Fig. 1I, the MYB gene transcript level was increased in the *ahs1* mutant, whereas the transcript level of the unknown gene was not altered (data not shown). The results suggest that the ABA hypersensitivity of *ahs1* mutant may result from the activation of the *AtMYB52* gene.

#### Expression pattern and subcellular localization of *AtMYB52*

We determined the tissue-specific expression pattern of *AtMYB52* by coupled reverse transcription and polymerase chain reaction (RT-PCR). As shown in Fig. 2A, *AtMYB52* was more abundantly expressed in roots and siliques than in flowers and leaves. Similar RT-PCR analysis indicated that the expression of *AtMYB52* in seedlings was slightly induced by ABA and high salt treatments (Fig. 2B). The expression patterns are in good agreement with those available in the public database ([www.arabidopsis.org](http://www.arabidopsis.org)). To explore the expression pattern of *AtMYB52* in detail, we prepared transgenic plants harboring an *AtMYB52* promoter-GUS fusion construct and investigated the promoter activity by histochemical GUS staining of the transgenic plants. Figure 2C shows that the *AtMYB52* promoter is active in roots of seedlings. In mature plants, promoter activity was observed in stems, flowers (i.e., receptacle, stigma, style and anthers) and siliques. Consistent with the RT-PCR result, stronger GUS staining was observed following ABA and salt treatments in seedlings, especially in the basal part and the maturation zone of lateral roots (Fig. 2D).

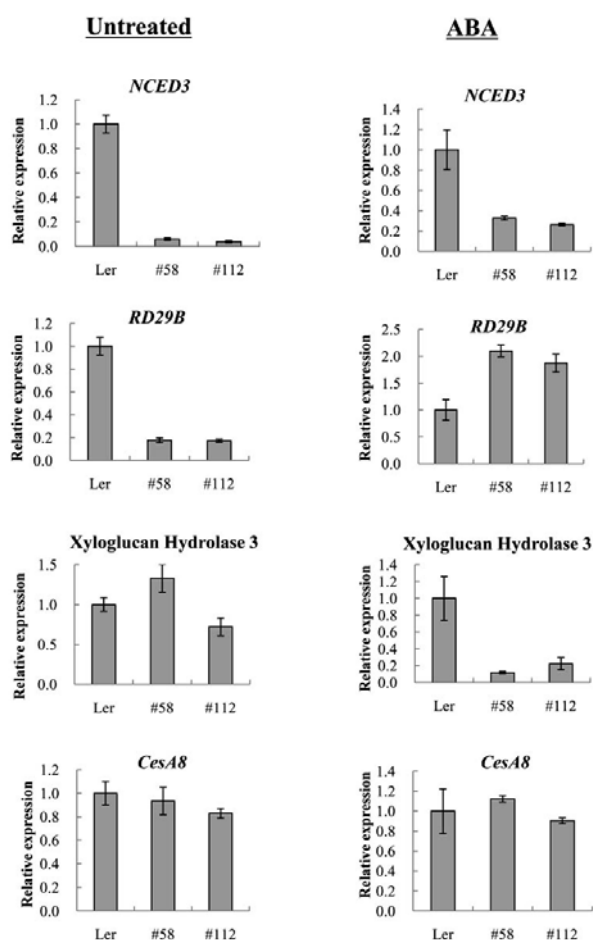
To examine subcellular localization of *AtMYB52*, an *AtMYB52*-YFP fusion protein construct was prepared and introduced into tobacco leaf cells by Agrobacterium infiltration (Voinnet et al., 2003). The *AtMYB52* localization was then investigated by fluorescence microscopy. As shown in Fig. 2E, the YFP signal was localized in the nucleus. Thus, our results indicated that *AtMYB52* was nuclear-localized.

#### Generation and phenotype analysis of *AtMYB52* overexpression lines

To confirm that the *ahs1* phenotype resulted from the overexpression of *AtMYB52*, we generated *AtMYB52* overexpression (OX) lines by expressing the *AtMYB52* gene under control of the strong CaMV 35S promoter. Although Col-0 was used in the construction of the activation tagged lines, we used Ler for the construction of *AtMYB52* overexpression lines because most of the genetic studies associated with ABA signaling were conducted in a Ler background. We recovered seven T3 generation homozygous lines and, after preliminary analysis, two representative lines were selected for further phenotype analyses.

As shown in Fig. 3A, the *AtMYB52* OX lines exhibited dwarfism that was dependent on the *AtMYB52* expression levels. However, similar to *ahs1*, the germination rates and overall growth of the OX lines were normal other than the dwarfism.

Because *ahs1* was hypersensitive to ABA, we examined whether *AtMYB52* OX lines were hypersensitive to ABA. We first scored the ABA effect on overall plant growth by growing the plants in the continual presence of ABA. The result (Fig. 3B) showed that growth of the OX lines was more severely inhibited by ABA than wild type plants. For example, at 0.5  $\mu\text{M}$  ABA, root growth of the transgenic seedlings was severely inhibited and shoot development of many seedlings was arrested. By contrast, growth of wild type plants was marginally affected by the low concentration of ABA. In the presence of 0.75  $\mu\text{M}$  ABA, the shoot and root growth of the transgenic seedlings was almost completely arrested, whereas wild type plants still grew, albeit at a lower rate (Fig. 3B). We next investigated shoot development at the seedling establishment stage by examining the efficiency of cotyledon greening. As shown in Fig. 3C, the cotyledons of most (~95%) of the wild type seedlings turned green at 0.5  $\mu\text{M}$  ABA. With the *AtMYB52* OX lines, green cotyledons were observed in 60% (#58) or 10% (#112) of the seedlings, respectively, indicating that transgenic shoot development was



**Fig. 5.** Expression analysis of putative *AtMYB52* target genes. Expression of ABA-responsive or cell wall biosynthesis genes in the transgenic lines was determined by Real-Time RT-PCR. RNA was prepared from plants treated with 100  $\mu$ M ABA for 4 h (ABA) or from untreated plants (Untreated). Reactions were performed in duplicate and the small bars indicate the standard errors.

more sensitive to ABA inhibition. As another indicator of ABA sensitivity, we conducted a root elongation assay. The result (Fig. 3D) showed that root growth of the transgenic lines was more severely inhibited by ABA than that of the wild type plants at all doses of ABA. Thus, overexpression of *AtMYB52* conferred ABA hypersensitivity during postgermination growth.

#### Drought and salt tolerance of *AtMYB52* OX lines

The results presented above suggested that *AtMYB52* affects ABA sensitivity. Because ABA mediates various abiotic stress responses (Xiong et al., 2002), especially drought response, we investigated whether *AtMYB52* overexpression affected drought tolerance. Three-week-old plants were subjected to water-deficit conditions by withholding water for two weeks. Subsequently, the plants were re-watered and their survival rates were determined. Figures 4A and 4B show that 75% of the *AtMYB52* OX line #58 survived the treatment, whereas the wild type survival rate was less than 3%. Similarly, the survival rate of another *AtMYB52* OX line, #112, was 81%, whereas that of the wild type was 10%. Thus, both *AtMYB52* OX lines exhibited higher survival rates than wild type plants, indicating that

they are drought-tolerant.

It has been well established that ABA mediates salt response (Xiong and Zhu, 2002). Hence, we next examined the salt sensitivity of the *AtMYB52* OX lines. Figure 4C shows that shoot development of the transgenic plants was more extensively inhibited by the salt. For example, green cotyledons were observed in more than 95% of the wild type seedlings in the presence of 125 mM NaCl. In contrast, green cotyledons were developed in only 50% (#58) and 20% (#112), respectively, of the transgenic seedlings (Fig. 4D). At 150 mM NaCl, shoot development of the *AtMYB52* OX lines was almost completely inhibited, whereas green cotyledons were observed in 95% of the wild type plants. Thus, shoot development of the *AtMYB52* OX lines was hypersensitive to salt. We also tested the effect of mannitol on seedling growth, but no difference between the OX lines and wild type plants was detected (not shown). The results imply that *AtMYB52* overexpression did not affect osmotic response and that the salt hypersensitivity of the transgenic lines may have been caused by altered ionic response rather than from changes in general osmotic response.

#### Target genes of *AtMYB52*

Because *AtMYB52* OX affected ABA and stress responses, we compared the expression levels of a number of ABA-responsive or ABA biosynthetic genes in the transgenic and wild type plants by Real-Time RT-PCR (Fig. 5). Among the genes examined, the expression levels of *NCED3* and *RD29B* were reduced in the *AtMYB52* OX lines under the normal growth condition. Because *AtMYB52* activity might be modulated posttranslationally by ABA, we also carried out similar experiments using RNA isolated from ABA-treated samples. Figure 5 shows that the relative *RD29B* expression levels in the transgenic lines were enhanced after ABA treatment, although *NCED3* expression was still lower than the wild type level. As mentioned earlier, *AtMYB52* is known to regulate cell wall biosynthesis (Zhong et al., 2008). Therefore, we determined the expression levels of two cell wall biosynthetic genes, *CesA8* and the xyloglucan hydrolase 3 gene. We did not observe any significant changes in their expression levels under the normal condition. However, after ABA treatment, the expression levels of the xyloglucan hydrolase 3 gene in the transgenic lines were lower than that in the wild type.

#### DISCUSSION

Several MYB class transcription factors are known to be involved in ABA and stress responses in Arabidopsis. *AtMYB2* regulates a subset of ABA-responsive genes, and its overexpression enhances ABA sensitivity (Abe et al., 2003). Similarly, *AtMYB15*, *AtMYB44* and *AtMYB96* are involved in ABA and drought responses (Ding et al., 2009; Jung et al., 2008; Seo et al., 2009), whereas *AtMYB41* regulates osmotic response (Lippold et al., 2009). In this study, we showed that overexpression of *AtMYB52* conferred ABA hypersensitivity, drought tolerance and salt sensitivity, which indicates that *AtMYB52* also is involved in ABA and stress responses.

As mentioned before, other studies have shown that *AtMYB52* is one of the downstream regulators of cell wall biosynthesis. Specifically, its overexpression affects the expression of several cell wall biosynthesis genes, although it does not influence secondary wall thickness, and dominant repression of its expression results in a reduction in secondary cell wall thickening (Ko et al., 2009; Zhong et al., 2008). Here, we demonstrated that *AtMYB52* is involved in ABA response during postgermination growth. Thus, our observations raise an interesting

possibility that ABA hypersensitivity of the *AtMYB52* OX lines may be associated with changes in cell wall architecture. It has been reported that ABA inhibits seed germination by inhibiting cell-wall loosening and expansion (Gimeno-Gilles et al., 2009). On the other hand, cell wall arabinan is essential for guard cell function (Jones et al., 2003; 2005) and seedling growth (Gomez et al., 2009). Therefore, it can be speculated that *AtMYB52* overexpression disturbed the biosynthesis of secondary cell wall components, which, in turn, caused a decrease in cell expansion, cell division/growth or stomatal movement. The decrease in the expression of the xyloglucan hydrolase3 gene in the presence of ABA (Fig. 5) that was observed herein supports this hypothesis. Alternatively, it is possible that *AtMYB52* affects the expression of genes involved in ABA metabolism or ABA response. The results shown in Fig. 5 indicate that expression of the ABA biosynthetic gene *NCED3* and the ABA-regulated gene *RD29B* was affected in the *AtMYB52* OX lines. More extensive expression and physiological analyses will be necessary to elucidate the molecular mechanism underlying the ABA-hypersensitivity of *AtMYB52* OX lines.

In summary, we isolated an ABA-hypersensitive mutant *ahs1* by activation tagging. *AtMYB52* was activated in the mutant, and a recapitulation experiment showed that *AtMYB52* overexpression conferred ABA-hypersensitivity during postgermination growth and enhanced drought tolerance of seedlings. Others have demonstrated that *AtMYB52* is involved in cell wall biosynthesis. Thus, our results suggest a possible connection between cell wall biosynthesis and ABA-dependent growth regulation of seedlings.

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