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Molecules and Cells

An ARIA-Interacting AP2 Domain Protein Is a Novel Component of ABA Signaling

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ADAP is an AP2-domain protein that interacts with ARIA, which, in turn, interacts with ABF2, a bZIP class transcription factor. ABF2 regulates various aspects of the abscisic acid (ABA) response by controlling the expression of a subset of ABA-responsive genes. Our expression analyses indicate that ADAP is expressed in roots, emerging young leaves, and flowers. We found that *adap* knockout mutant lines germinate more efficiently than wild-type plants and that the mutant seedlings grow faster. This suggests that ADAP is involved in the regulation of germination and seedling growth. Both germination and post-germination growth of the knockout mutants were partially insensitive to ABA, which indicates that ADAP is required for a full ABA response. The survival rates for mutants from which water was withheld were low compared with those for wild-type plants. The result shows that ADAP is necessary for the response to stress induced by water deprivation. Together, our data indicate that ADAP is a positive regulator of the ABA response and is also involved in regulating seedling growth. The role of ADAP is similar to that of ARIA, which is also a positive regulator of the ABA response. It appears that ADAP acts through the same ABA response pathway as ARIA.

INTRODUCTION

Abscisic acid (ABA) is a major plant hormone that controls various aspects of plant growth and development (Finkelstein et al., 2002). The hormone regulates seed maturation and prevents embryos from precocious germination. During seedling growth, ABA mediates adaptive responses to various types of abiotic stress, such as drought, high salinity, and extreme temperatures (Xiong et al., 2002).

Numerous genetic and biochemical studies have been carried out to identify regulatory components of the ABA response. Consequently, a large number of ABA signaling components have been reported, including several transcriptional regulators (eg, bZIP, AP2/ERF, MYB, NAC, and WRKY family proteins) (Hirayama and Shinozaki 2007; Yamaguchi-Shinozaki and Shinozaki, 2005). Some of the transcription factors are negative regulators, including ABR1 (Pandy et al., 2005) and AtERF7 (Song et al., 2005). However, most of them are positive regulators of the ABA response, including several bZIP proteins (Foster et al., 1994; Landschulz et al., 1988) referred to as ABFs (Choi et al., 2000). ABFs (also known as AREBs) (Uno et al., 2000) have been isolated based on their ability to bind to the G-box type ABA response element (ABRE) (Busk and Pages, 1998) containing the CACGTGGC consensus sequence. ABFs/AREBs have been shown to control the ABA response and the response to abiotic stress *in vivo* (Kang et al., 2002; Kim et al., 2004a; 2004b; Sakuma et al., 2006a; 2006b). In particular, ABF3 and ABF4/AREB2 are essential for normal ABA and stress responses. ABF2/AREB1, on the other hand, is necessary for seedling growth regulation and glucose response; additionally, its constitutively active form is involved in the response to stress (Sakuma et al., 2006a).

We are interested in identifying signaling components that modulate the activity of ABFs/AREBs. Toward this end, we carried out yeast two-hybrid screens and showed that several ABF-interacting proteins, including a calcium-dependent protein kinase (Choi et al., 2005) and an armadillo (ARM) repeat protein (Kim et al., 2004c), are novel components of the ABA signaling. The ARM repeat protein, which is an ABF2-interacting protein known as ARIA (Arm Repeat Protein Interacting with ABF2), possesses another conserved sequence motif, the BTB/POZ (broad complex, tramtrak, and bric-a-brac/poxvirus and zinc finger) domain (Bardwell and Treisman, 1994; Collins et al., 2001) in its C-terminal region. ARIA overexpression lines are ABA- and salt-hypersensitive during germination but salt-insensitive during subsequent seedling growth. By contrast, an ARIA knockout mutant exhibits ABA and glucose insensitivity.

To identify ABA signaling components that modify ARIA activity, we carried out two-hybrid screens and isolated several ARIA-interacting proteins. We report that an ARIA-interacting protein containing two AP2 domains is involved in the ABA response and the response to stress. An *in vivo* functional analysis of this protein, which is named ADAP (ARIA-interacting double AP2-domain protein), suggests that ADAP is a novel ABA-signaling component that regulates seed germination, seedling growth, and the ABA response, as well as the response to salt, drought, or both.

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MATERIALS AND METHODS

Two-hybrid screen

Two-hybrid screens were carried out as described previously (Kim et al., 2004c; Choi et al., 2005), with minor modification: the bait construct was prepared using the vector pPC62LexA, which was prepared by replacing the GAL4 DB of pPC62 (Chien et al., 1991) with LexA DB. The entire coding region of ARIA was prepared by polymerase chain reaction (PCR) using the primer set 5′-ATGCCGTCGGAGATTGTTGA-CAG-3′ and 5′-ttttccttttgcggccgcAATCTTATGTAGATCC ATG-AACATC-3′, digested with *Not*I and cloned into the *Sma*I-*Not*I sites of pPC62LexA. The bait construct was introduced into L40 (MATα, his3Δ200, trp1-901, leu2-3112, ade2, LYS2::[LexAop (x4)-HIS3], URA3::LexAop[x8]-LacZ, GAL4) (Invitrogen, USA). Transformation of the reporter yeast and the screening of the resulting transformants were carried out as described (Choi et al., 2005; Kim et al., 2004c).

Arabidopsis growth

Arabidopsis thaliana (Ecotype *Landsberg erecta*, L*er* and *Columbia*, Col-0) was grown aseptically or in soil at 22°C under long-day condition (16-h light/8-h dark cycle). For aseptic growth, seeds were treated with 70% ethanol for 1 min and with 30% house bleach for 5 min then washed 5 times with sterile water before plating on Murashige and Skoog Basal medium (MS medium) (Murashige and Skoog, 1962). The MS medium, supplemented with 1.0% (w/v) sucrose, was solidified with 0.8% (w/v) phytoagar (Duchefa, USA), and ABA, salts, glucose, or mannitol was added to the medium as needed. For germination tests, seeds collected at the same or similar times were used. For root growth measurements, plants were germinated and grown in the vertical position.

Vector constructs

Unless stated otherwise, DNA was manipulated according to the standard procedure (Sambrook and Russell, 2001). The *35S-ADAP* constructs were prepared by replacing the β-glucuronidase (GUS) coding region of pBI121 (Jefferson et al., 1987) with the coding region of *ADAP*. The GUS sequence was removed after *Sac*I-*Sma*I digestion, and the remaining portion of pBI121 was self-ligated after T4 DNA polymerase treatment. The vector was then digested with *Xba*I, treated with Klenow fragment, and ligated with the *ADAP* coding region (1.03 kb), which was prepared by PCR using the primer set, 5′-CGCG-GATCCTA TGTTCATCGCCGTC GAAGTTTC-3′ and 5′-ACTTTAGCAATC-ATTTAACTCGCTG-3′. Intactness of the junction sequence and the *ADAP* coding region was confirmed by DNA sequencing.

To prepare the *ADAP* promoter-*GUS* reporter fusion construct, the promoter region of *ADAP* (1.8 kb) was prepared by PCR using *Arabidopsis* (ecotype Col-0) genomic DNA as a template and the primers 5′-attaaagcttGCATCTCTATATACGCAGCCG-ACA-3′ and 5′-AAGGATGAG AAATTGATGGAATGGT-3′. The amplified fragment was digested with *Hind*III and cloned into the *Hind*III-*Sma*I sites of the pBI101.2 vector. The promoter and the junction sequences were confirmed by DNA sequencing.

Arabidopsis transformation

Transformation of *Arabidopsis* was carried out according to the vacuum infiltration method (Bechtold and Pelletier, 1998) using *Agrobacterium tumefaciens* (stain GV3101). T3- or T4-generation homozygous plants were used for phenotype analysis.

Expression analysis

RNA was isolated using the RNeasy plant mini kit (Qiagen, USA). For RNA gel blot analysis, 10 μg of total RNA was fractionated on 1.1% formaldehyde-agarose gel, transferred to nylon membrane (Hybond N+, Amersham AIPK Biotech), and fixed using the Stratagene UV Crosslinker (Model 2400). Loading of equal amounts of RNAs was confirmed by ethidum bromide staining. Hybridization was carried out as described previously (Kang et al., 2002). For reverse-transcriptase (RT)-PCR analysis, 0.5 μg of total RNA was processed using the Access RT-PCR system (Promega, USA) according to the supplier's instructions. First-strand cDNA synthesis was carried out at 45°C for 50 min, and amplification was carried out at 94°C for 10 s, 50°C for 15 s, and then 68°C for 1.5 min.

Transcription assay

Full-length *ADAP* or *ADAP* fragments (Fig. 2A) lacking various portions of *ADAP* were prepared by PCR and cloned into the *Pst*I *and Xba*I sites of the pPC62/LexA (DB) vector. Primer sequences are available on request. The intactness of the junction sequence of the fusion constructs was confirmed by restriction enzyme digestion and DNA sequencing.

For the filter lift β-galactosidase assay, transformants were streaked onto a synthetic complete supplement (SC)-Leu-Ura medium, grown at 30°C for 5 d, and lifted from the medium using filter papers (Whatman No 5). The filters were immersed into liquid nitrogen for 30 s, thawed at room temperature for 5 min, and placed on filter papers soaked with an X-gal (0.5 mg/ml) solution. The reactions were stopped after 2 to 4 hours at 30°C. For each construct, 3 independent transformants were assayed.

For the liquid β-galactosidase assay, colonies of yeast transformants were grown in an SC-Leu-Ura medium to an A_{600} of approximately 0.5. The cultures were diluted 4 times with fresh media and grown further for 3 h. After the A_{600} was measured, 1.5-ml aliquots of the cultures briefly underwent centrifugation in a microfuge. The pellets were resuspended in 0.5 ml of Z-buffer (60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCL, and 1 mM $MgSO_4{\cdot}7H_2O$) without β-mercaptoethanol, microfuged briefly again, and resuspended in 0.3 ml of Z-buffer. One third of the suspension was transferred to a fresh tube, placed in liquid nitrogen until frozen, and thawed in a 37°C water bath. The freeze-thaw cycle was repeated 2 more times. Afterward, 0.7 ml of Z-buffer with β-mercaptoethanol and 0.16 ml of O-nitrophenyl-β-D-galactopyranoside (ONPG) (4 mg/ml in Z-buffer) were added to start the reaction. Incubation was continued at 30°C until the color changed to yellow. Reactions were stopped by the addition of 0.4 ml of 1 M Na_2CO_3. The mixtures underwent microcentrifugation for 10 min to remove cell debris, and the A_{420} was measured. Four independent transformants were assayed for each construct, and the β-galactosidase activity was expressed in Miller units.

Phenotype analysis of knockout mutants

Seeds of the knockout mutant line (SALK_125491) were obtained from the *Arabidopsis* stock center. Stock seeds were germinated and grown in soil then seeds were harvested from individual plants. After testing the segregation by kanamycin resistance, homozygous sublines exhibiting 100% kanamycin resistance were established from plants whose progeny segregated with a 3:1 ratio of kanamycin-resistant and kanamycin-susceptible seeds. DNA was isolated from the selected lines, and T-DNA insertion in the annotated positions was confirmed by PCR and subsequent sequencing of the amplified fragments.

Drought test

Nine-day-old plants were grown in soil without water for 2

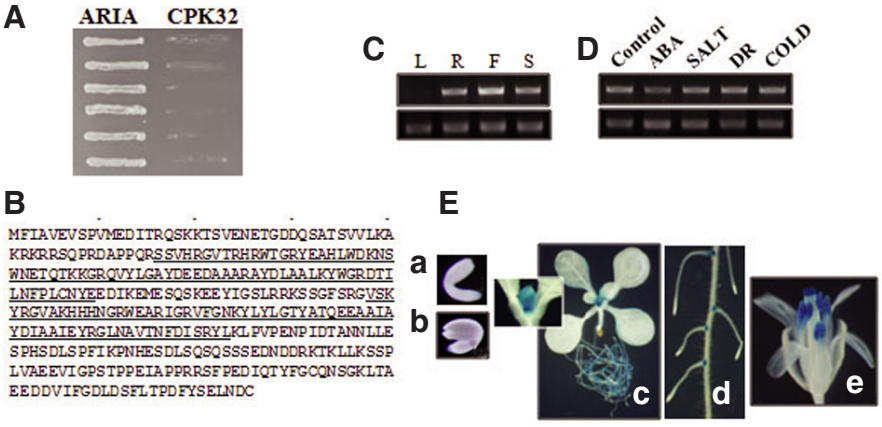

Fig. 1. ADAP isolation and expression patterns. (A) The specificity of interaction between ARIA and ADAP was determined using a two-hybrid assay with ARIA or an unrelated protein (AtCPK32) as bait. (B) Deduced amino-acid sequence of ADAP. AP2/ERF domains are underlined. (C) Tissue specificity of *ADAP* expression. RNA was isolated from various tissues of wild-type plants that were grown under normal conditions, and the *ADAP* expression was determined using RT-PCR. *L,* leaves from 3-week-old plants; *R,* roots from 3-week-old plants; *F,* flowers. *S,* immature siliques. (D) Induction patterns of *ADAP* expression. RNA was isolated from 3-week-old seedlings treated with 100 μM ABA (4 h), 250 mM NaCl (4 h), cold (4°C at 24 h), or dehydration (water withheld for 2 weeks), and ADAP expression was determined using RT-PCR. *Control,* RNA from untreated seedlings. (E) Histochemical GUS staining of transgenic plants transformed with an *ADAP* promoter-*GUS* reporter construct. *a,* an immature embryo; *b,* an mature embryo; *c,* a 9-day-old seedling; *d,* root of 9-day-old seedling; *e,* a flower. The inset in c shows a leaf emerging from the apical region of the shoot.

weeks. The same number of plants were grown in each container to minimize experimental variations. The drought tests were repeated 2 to 4 times.

Histochemical GUS staining

An *in situ* assay of GUS activity was carried out as described by Jefferson et al. (1987). In preparation for staining, whole plants grown aseptically in 1/2 MS medium or in soil were immersed in 1 mM 5-bromo-4-chloro-3-indolyl-β-glucuronic acid solution (Duchefa) in 100 mM sodium phosphate (pH 7.0), 0.1 mM EDTA, 0.5 mM ferricyanide, 0.5 mM ferrocyanide, and 0.1% Triton X-100. After a vacuum was applied to the plants for 5 min, they were incubated at 37°C until sufficiently stained. Chlorophyll was cleared from the plant tissue by immersing them sequentially in 50%, 70%, and 95% ethanol. The plants were then photographed through a dissecting microscope (Olympus SZX-ILLD200).

RESULTS

Isolation of ADAP

Two-hybrid screens were carried out to isolate genes encoding ARIA-interacting proteins. Using the entire coding region of ARIA as bait, we obtained a number of positive clones from the screen of 2.6 million yeast transformants. Analysis of the positive clones showed that one of them (clone #24) (Fig. 1A) encodes a protein with 2 AP2/ERF domains (Nakano et al., 2006) (Fig. 1B). This protein, which we named ARIA- interacting Double AP2 domain Protein, At1g16060 (ADAP), interacts with ARIA but not with an unrelated protein (Fig. 1A). Outside the AP2 domain regions, ADAP does not show significant homology with any of the AP2 family proteins.

Adap expression pattern

Tissue-specific *ADAP* expression pattern were determined by RT-PCR. *ADAP* expression was detected in the roots, flowers, and siliques (Fig. 1C). We also examined ABA- and stress-induction patterns of *ADAP* expression using RT-PCR. Figure 1D shows that the *ADAP* transcript level was not changed significantly by ABA or by the application of various types of stresses.

To investigate the temporal and spatial expression patterns of *ADAP*, we generated transgenic *Arabidopsis* plants harboring an *ADAP*-promoter-*GUS* reporter construct and carried out histochemical GUS staining to determine promoter activity. GUS activity was not observed in embryos (Figs. 1E-a and 1E-b), but strong GUS activity was detected in the leaves emerging from young seedlings (Fig. 1E-c). GUS activity was also detected in the roots, especially in the lateral root primordia and in the base regions of the lateral roots (Fig. 1E-d). Among the reproductive organs, the anthers and stigma displayed strong GUS activity (Fig. 1E-e).

Transcriptional activity of ADAP

To determine whether ADAP possesses transcriptional activity, we cloned full-length or partial ADAP fragments (Fig. 2A) into a yeast expression vector pPC62/LexA (see "Materials and Methods") in-frame with the LexA DNA-binding domain. Each construct was then introduced to a yeast strain (L40) containing a *lacZ* reporter gene, which has a LexA DB in its promoter.

As shown in Fig. 2B, reporter activity, which was much higher than that of the vector (i.e., pPC62/LexA) alone, was observed with the full-length ADAP (#1) and with 3 N-terminal deletion constructs (#2, 3, and 4). By contrast, no β-galactosidase activity was detected with the 2 C-terminal deletion constructs (#5, #6). In the quantitative β-galactosidase assay, the 4 constructs (#1-#4) that displayed transcriptional activity in the filter-lift assay exhibited much higher reporter activity than was observed for the control vector without any inserts (Fig. 2C). In particular, the highest level of activity was observed with the construct harboring the C-terminal region only. By contrast, no enzyme activity was detected using constructs lacking the C-terminal region. Taken together, our results demonstrate that ADAP possesses transcriptional activity and that the activity resides in its C-terminal portion.

Growth phenotypes of ADAP overexpression lines

To investigate the *in vivo* function of ADAP, we generated transgenic *Arabidopsis* plants overexpressing *ADAP* under the control of the cauliflower mosaic virus 35S promoter. T3 or T4 homozygous lines were recovered, and after preliminary analysis, 3 representative lines were chosen for detailed analysis (Fig. 3A).

The overall growth patterns of the *35S-ADAP* plants were

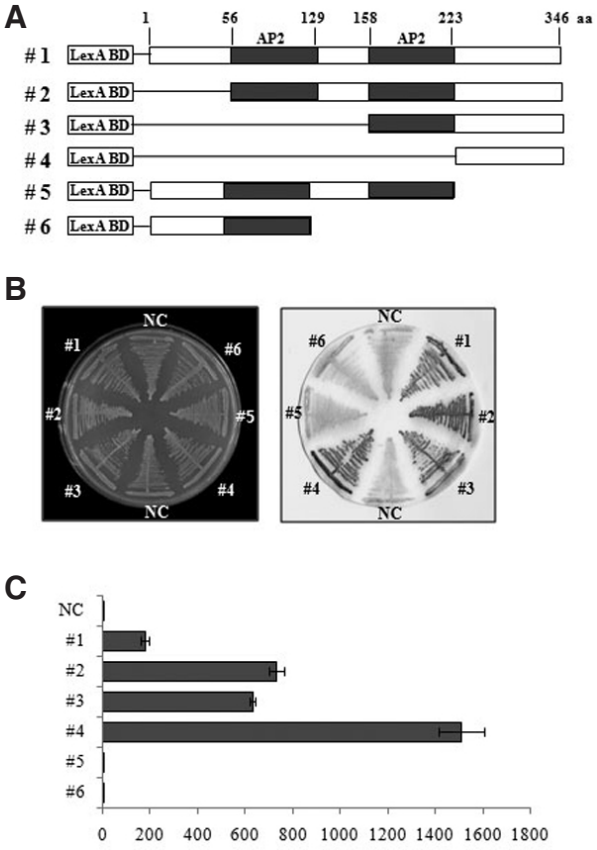

Fig. 2. Transcription assay of ADAP. The transcriptional activity of ADAP was determined using a yeast assay system in which ADAP was expressed in yeast containing a *lacZ* reporter gene. (A) ADAP constructs. Six constructs were prepared containing various portions of ADAP. (B) Filter lift β-galactosidase assay was performed. (C) The liquid β-galactosidase assay was performed using ONPG as a substrate. β-Galactosidase activity was then assayed and expressed in Miller units. For each construct, 4 different transformants were assayed. NC; control vector without any inserts. Bars represent standard error.

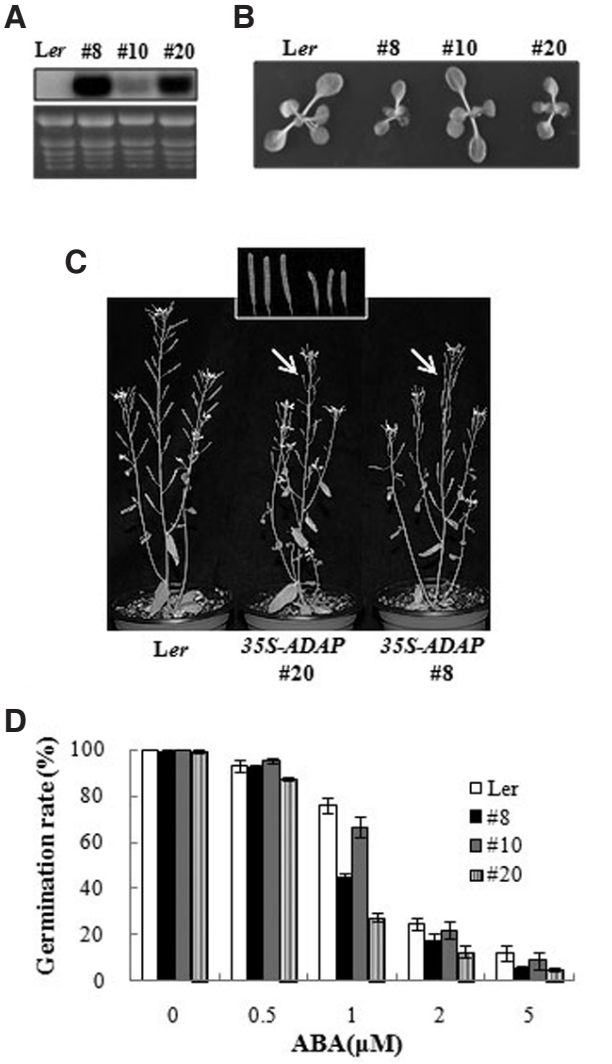

Fig. 3. Growth phenotypes and ABA sensitivity of *35S-ADAP* transgenic plants. (A) Level of expression of *ADAP* in transgenic lines determined by RNA gel blot analysis. The bottom panel shows the ethidium-bromide-stained gel as a loading control. Each lane contained 10 μg of total RNA. (B) Shoots of 12-day-old seedlings grown on MS. (C) Mature plants grown in soil. *Left,* 39-day-old plants grown in soil. *Right,* 7-week-old plants grown in soil. The inset and the arrow show siliques and erect siliques, respectively. (D) ABA dose-response analysis of germination. Seeds were plated on medium containing various amounts of ABA, and germination (radicle emergence) was scored 2 days after plating (triplicates, n = 50 each). The small bars represent standard error.

similar to those of wild-type plants. However, the ADAP OX lines exhibited several observable phenotypes: (1) the *ADAP* overexpression lines exhibited growth retardation that varied with the level of *ADAP* expression (Figs. 3A and 3B); (2) both the number and the size of the siliques of the *35S-ADAP* transgenic plants were smaller than for their wild-type counterparts (Fig. 3C); and (3) the siliques of the *35S-ADAP* transgenic plants were erect in most of the plants.

ABA response of ADAP overexpression lines

To determine whether overexpression of ADAP affects ABA sensitivity, we determined the germination rates for *35S-ADAP* transgenic seeds in the presence of various concentrations of ABA. We did not observe a marked difference in ABA response at a low concentration (0.5 μM) of ABA. However, at higher concentrations of ABA, greater inhibition of germination was observed in the transgenic seeds. At an ABA concentration of 1 μM, for example, the germination rate for wild-type seeds was 76% of the rate observed in the absence of ABA (Fig. 3D). By contrast, the germination rates for *ADAP* overexpression lines were 66% (# 10 line), 28% (# 20 line) and 46% (# 8 line). Thus, the germination assay revealed that *35S-ADAP* transgenic seeds were hypersensitive to ABA during germination.

Response of ADAP overexpression lines to salt

To investigate the salt sensitivity of *35S-ADAP* plants, seeds of wild-type and *35S-ADAP* plants were germinated and grown in MS medium containing various concentrations of salt. We did not observe clear differences in the response to salt during the germination stage in *35S-ADAP* plants (data not shown). However, postgermination growth of *35S-ADAP* plants at the cotyledon greening stage displayed partial insensitivity to salt. As

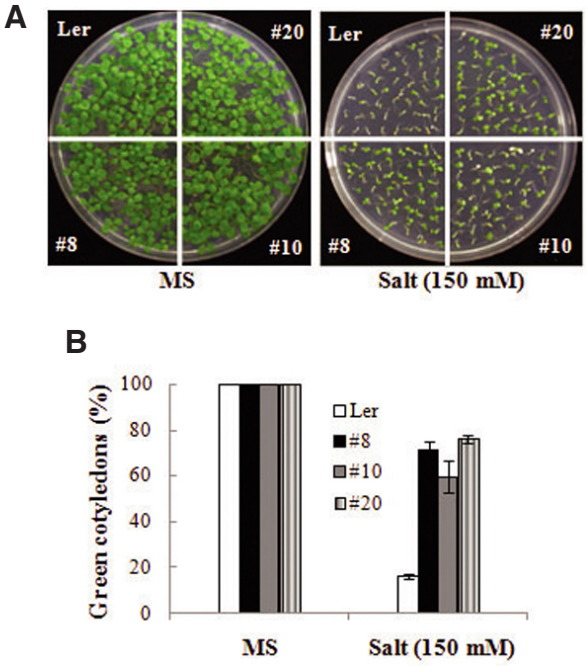

Fig. 4. Salt sensitivity of *35S-ADAP* plants. Response to salt during cotyledon greening. Seeds were germinated and grown on MS medium containing 150 mM NaCl for 10 d, and plants with green cotyledons were counted (triplicates, n = 50 each). (A) A photograph showing seedlings. (B) Percentage of seedlings with green cotyledons. The small bars represent standard errors.

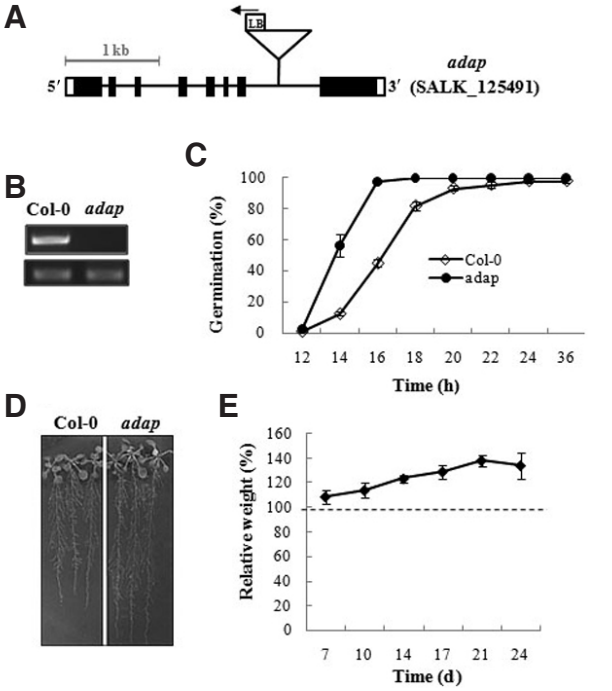

Fig. 5. Germination and growth of an *adap* knockout mutant. (A) Schematic diagram of the T-DNA insertion site. Black rectangles represent exons; lines represent introns. T-DNA is inserted in the 7th intron in SALK_125491. LB: T-DNA left border. (B) Level of *ADAP* expression in the mutant was determined by RT-PCR. Actin was used as an internal control (lower panel). (C) Germination assay. Seeds were plated on MS medium after 5 days of cold treatment at 4°C, and germination (fully emerged radicle) was scored at various time points (triplicates, n = 50 each). The small bars represent standard errors. (D) Seedlings grown on 1/2 MS medium for 2 weeks. (E) Relative weight of aerial parts of soil-grown plants compared with that of Col-0 plants. The data points represent the mean of 6 determinations (n = 10 each). The small bars represent standard errors.

presented in Fig. 4, cotyledons in 60% to 80% of the *35S-ADAP* seedlings turned green in MS medium containing 150 mM NaCl, whereas fewer than 20% of the wild-type cotyledons turned green.

Growth phenotypes of an *adap* knockout mutant

To gain further insight into the *in vivo* function of ADAP, we investigated the *adap* mutant phenotypes. An insertion mutant line into which T-DNA is inserted in the 7th intron of *ADAP* (Fig. 5A) was acquired from the *Arabidopsis* stock center. The expression of *ADAP* was abolished completely in this mutant (Fig. 5B).

A germination assay (Fig. 5C) revealed that the seeds of mutant lines germinated more efficiently than wild-type seeds, i.e., they germinated several hours earlier than wild-type seeds under normal growth conditions. Seedlings of the knockout line also grew faster than wild-type seedlings (Fig. 5D). As a result, the shoots of the 3-week-old *adap* mutant plants grown in soil weighed approximately 40% more than the wild-type shoots (Fig. 5E). However, the difference was observed only with seedlings; mature mutant plants were of the same size as wild-type plants. These results indicate that ADAP is a negative regulator of seed germination and seedling growth under normal growth conditions.

ABA response of *adap* mutant plants

To investigate the ABA sensitivity of *adap* mutants, we examined seedling growth in media containing ABA. As shown in Fig. 6A, little difference in growth was observed between mutant and wild-type seedlings in the medium lacking ABA. However, the mutant plants grew larger when seeds were germinated and grown in the presence of 0.75 μM ABA, which suggests that they are less sensitive to ABA inhibition than wild-type plants.

To investigate sensitivity to ABA in more detail, we conducted an ABA dose-response analysis of germination. The assay (Fig. 6B) showed that mutant seed germination was less sensitive to ABA inhibition than wild-type seeds. For example, at an ABA concentration of 2 μM, the germination rate for wild-type plants was 47% of that in controls, whereas *adap* mutants retained 83% of their germination rates, indicating that germination was less sensitive to ABA inhibition in *adap* mutants.

We also carried out an ABA dose-response analysis of post-germination growth. Figure 6C shows that shoot development (i.e., cotyledon greening, cotyledon expansion or both) in *adap* mutant plants was less sensitive to ABA inhibition than wild-type plants. Similarly, higher rates of root elongation were observed for *adap* mutants than for wild-type seedlings (Fig. 6D). The difference was not great; nonetheless, it was observed consistently. Taken together, these results indicate that both germination and seedling growth of *adap* mutants are partially insensitive to ABA.

Response of adap mutant plants to stress

To examine the response of the *adap* mutant to stress, we

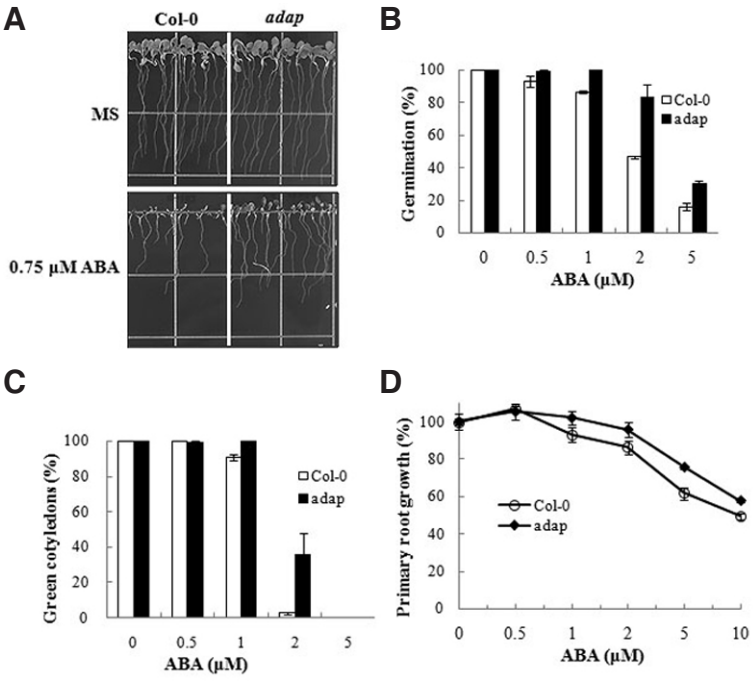

Fig. 6. ABA sensitivity of an *adap* knockout mutant. (A) Growth of an *adap* knockout mutant on MS medium containing 0.75 μM ABA. Seeds were germinated and grown for 6 d in a vertical position. (B) ABA dose-response analysis of germination. Seeds were plated on media containing various amounts of ABA after 5 d of cold treatment at 4°C, and germination (radicle emergence) was scored 2 d after plating (triplicates, n = 50 each). The small bars represent standard errors. (C) Seeds were germinated and grown on MS medium containing various concentrations of ABA for 7 d before plants with green cotyledons were counted (triplicates, n = 50 each). The small bars represent standard errors. (D) ABA dose-response analysis of primary root elongation. Seeds were germinated on MS medium for 3 d and transferred to media containing various amounts of ABA. The primary root length was measured 5 d after the transfer. Elongation rates relative to those seen in the ABA-free control medium (triplicates, n = 6 each) are presented. The small bars represent standard errors.

determined the salt and the drought tolerance of the mutant plants. The salt tolerance test indicated that germination of the *adap* mutant seeds was less severely inhibited by a high-salt environment than in wild-type seeds (Fig. 7A). For example, germination rates for the wild-type seeds were 50% and 15% of the control rates at sodium chloride concentrations of 125 and 150 mM, respectively. By contrast, the germination rates for *adap* mutants were 95% and 70%, respectively. These results indicate that germination of *adap* mutants is partially insensitive to a high-salt environment.

To determine whether there is a role for ADAP in the response to drought, whole-plant survival rates were determined for the *35S-ADAP* plants and for the *adap* mutant plants under water-deficient conditions. We did not observe significant differences in survival rates among the *35S-ADAP* plants. However, survival rates for the *adap* mutant plants were lower than the wild-type rates. For example, the average survival rate of the *adap* mutant plants was 35% when they were deprived of water for 14 d, whereas 81% of the wild-type plants survived under the drought conditions. These results indicated that *adap* mutant plants were more sensitive to water-deficient conditions compared with wild-type plants.

DISCUSSION

The *Arabidopsis* genome contains 14 members of the AP2 family of proteins, which contains two AP2/ERF domains (Nakano et al., 2006; Sakuma et al., 2002). Three of these proteins regulate the development of the plant. AP2, for example, regulates flower development (Jofuku et al., 1994), ANT controls ovule development and floral organ growth (Elliot et al., 1996; Klucher et al., 1996), and PLT1 and PLT2 determine root stem cell niches (Aida et al., 2004). By contrast, WR1 controls seed oil accumulation (Cernac and Benning, 2004) and, consequently, affects seed germination (Cernac et al., 2006). The functions of the other AP2 family proteins remain to be determined. Several AP2 proteins from other species have also been reported. A *Brassica napus* AP2 protein, BBM, regulates embryo development (Boutilier et al., 2002), and maize AP2 proteins Gl15 and Ids1 regulate leaf epidermal cell identity and spikelet meristem fate, respectively (Chuck et al., 1998; Moose and Sisco, 1996).

Our results indicate that ADAP, an *Arabidopsis* AP2 family protein that interacts with ARIA, affects seedling growth and ABA response. In our studies, *adap* knockout mutant seeds germinated more efficiently than wild-type seeds, and their young seedlings grew faster under normal growth conditions. These results suggest that ADAP is involved in the regulation of germination and early seedling growth. In addition, the *adap* mutants were partially insensitive to ABA during germination and subsequent establishment and growth of seedlings (i.e., cotyledon greening, expansion, or both and root elongation). Together, our results indicate that ADAP is involved in seedling growth regulation under normal conditions and is necessary for ABA-mediated inhibition of germination and seedling growth.

Our expression analysis showed that the *ADAP* promoter was active in leaves emerging from young seedlings. The *ADAP* promoter was also very active in the lateral root primordia and in the base region of lateral roots. The expression pattern is consistent with the role of ADAP in regulating seedling growth. In addition, the *ADAP* promoter exhibited strong activity in the anthers and stigma. These results suggest that ADAP may play a role in the reproductive stage. This notion is supported by the reduced fertility of *ADAP* overexpresion lines. Unlike ARIA, however, *ADAP* expression could not be induced by ABA or a high-salt environment. It appears that *ADAP* expression is constitutive; thus, its activity might be modulated by other mechanisms, such as posttranslation modification or through its interaction with ARIA, which can be induced by both ABA and a high-salt environment.

Our transcription assay indicates that ADAP transcriptional activity resides in the C-terminal region, which suggests that it may act as a transcriptional activator. Consistent with this possibility, ADAP OX lines displayed several of the observable phenotypes mentioned in the Results section, i.e., minor growth retardation, reduced fertility, and altered ABA and stress re-

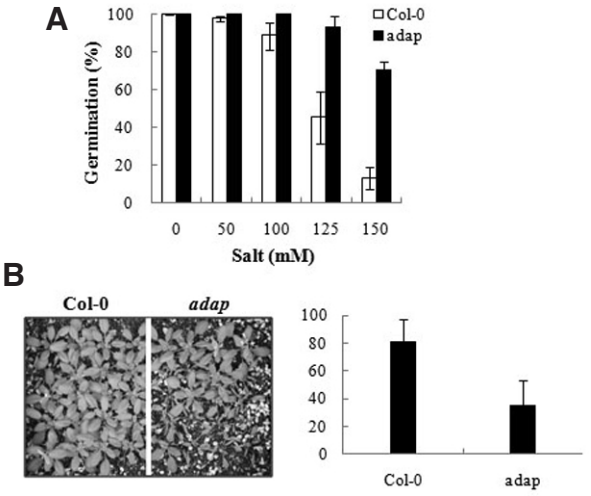

Fig. 7. Salt and drought tolerance of an *adap* knockout mutant. (A) Salt dose-response analysis of germination. Seeds were plated on medium containing various amounts of NaCl after 5 d of cold treatment at 4°C, and germination (radicle emergence) was scored 2 d after plating (triplicates, n = 50 each). The small bars represent standard errors. (B) Drought sensitivity of an *adap* mutant. Plants were grown on soil for 9 d, deprived of water for 14 d, and then re-watered. Left, photograph of plants 3 d after re-watering. Right, survival rates of plants. Experiments were done in duplicates (n = 16 each).

sponses. Unlike the *adap* knockout mutants, however, the ADAP OX lines did not exhibit significant changes in ABA sensitivity except during germination. This observation suggests that ADAP is necessary for a normal ABA response, but it alone is not sufficient for a full ABA response.

ADAP interacts with ARIA, which is a positive regulator of the ABA response. We do not know the molecular mechanism underlying this ARIA function yet, and it is difficult to predict the outcome of ADAP-ARIA interaction. However, given that (1) the expression patterns of ADAP partially overlap with those of ARIA (which is also highly expressed in the lateral roots, anthers, and stigma), and (2) the insensitivity to salt observed for the *ADAP* OX lines during postgermination growth and the insensitivity to ABA observed in knockout mutant lines were also observed with ARIA, we suggest that ADAP probably functions in the same ABA-response pathway as ARIA.

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