Dual role of SND1 facilitates efficient communication between abiotic stress signalling and normal growth in Arabidopsis

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Certain plant cells synthesize secondary cell walls besides primary cell walls. This biosynthesis is strictly controlled by an array of transcription factors. Here, we show that SND1, a regulator of cell-wall biosynthesis, regulates abscisic acid (ABA) biosynthesis to ensure optimal plant growth. In Arabidopsis, the lack of SND1 and its homolog NST1 leads to the deficiency of secondary cell walls, preventing snd1nst1 double mutant seedlings from growing upright. Compared to wild type seedlings, the snd1 knockout mutant seedlings accumulated less anthocyanin and exhibited low tolerance to salt stress. Compared to wild type seedlings, the snd1 knockout seedlings were more sensitive to salt stress. Although SND1 can bind to the promoter of Myb46, we observed that SND1 binds directly to the promoter of the ABI4 gene, thereby reducing ABA levels under normal growth conditions. Thus, plants adjust secondary cell wall thickening and growth via SND1. SND1 has a dual function: it activates the Myb46 pathway, fostering lignin biosynthesis to produce sufficient cell wall components for growth, while maintaining a low ABA concentration, as it inhibits growth. This dual function of SND1 may help plants modulate their growth efficiently.

In addition to primary cell walls, plant cells also have secondary walls, composed of cellulose, lignin, and other molecules. Because only certain types of plant cells can deposit secondary cell wall materials, including phenylpropanoid, during specific developmental phases, phenylpropanoid biosynthesis is strictly controlled by an array of genes, which have been targeted to modify lignin content in order to manipulate biomass composition, as well as plant tolerance to abiotic stress. Diverse transcription factors (TF) modulate various compounds in the phenylpropanoid biosynthesis pathway. AtMyb46 and its homologs AtMyb83, AtMyb58 and AtMyb63, play crucial roles in cell-wall biosynthesis. Furthermore, NAC (NAM, ATAF1/2, and CUC2)-domain TFs are also xylem-associated, and 105 NAC genes with numerous functions exist in the genome of Arabidopsis thaliana. Among these, the secondary wall-associated NAC domain protein 1 (SND1) is expressed in fibre-associated cells and plays a central role in fibre thickening.

Complex and multifaceted signalling cascades, accompanied by various cellular responses, are activated in plants to ensure their survival under conditions of abiotic stress. Plant cells produce osmoprotectants to compensate for the water loss caused by salt, drought, or cold stress. High salinity and drought may cause a marked imbalance in redox homeostasis, which drives plant cells to reorganize primary as well as secondary metabolism. In this process, plant hormones serve as crucial integrators that modulate complex developmental and stress signalling pathways. Among them, abscisic acid (ABA) enables Arabidopsis to adapt to detrimental conditions.

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conditions imposed by abiotic stress and often triggers the inhibition of plant growth, thereby re-directing nutrients for successful withstanding of the specific stress conditions.

Anthocyanins are recognized as part of the defence mechanism that plants use when challenged by stress. Indeed, they often accumulate in response to stress. We aimed to verify whether SND1, the master controller of cell-wall biosynthesis, has any role under plant stress, as expression of SND1 is known to affect the accumulation of lignins which are produced from the same precursor of anthocyanins. Herein we report that SND1 directly regulates ABA biosynthesis to procure best possible plant growth under salinity stress. Furthermore, we show that SND1 binds directly to the promoter of the ABI4 gene, leading to low levels of ABA under saline conditions. Our observations suggest that plants can adjust secondary cell-wall thickening and growth performance via this SND1 regulatory effect, which displays a dual function by thickening secondary walls, while concomitantly reducing ABA content when environmental conditions are favourable plant growth.

Results
Altered anthocyanin content in the snd1ko mutant and SND1-overexpressing line and SND1 was induced by abiotic stresses. In our previous study, we showed that several genes involved in flavonoid biosynthesis participate in plant abiotic stress tolerance. Plants accumulate a wide variety of flavonoids via phenylalanine through elaborate regulatory mechanisms. There are several junctions in this pathway, leading to the synthesis of different types of flavonoid compounds. For example, coumaroyl CoA, which is utilized to produce anthocyanins via various enzymes including chalcone synthase (CHS), can be converted into lignins by hydroxycinnamoyl transferase (HCT). Thus, the synthesis of anthocyanin likely affects the synthesis of lignin, which belongs to the flavonoid family. SND1 is essential for the synthesis of lignin, and thereby for the formation of secondary cell walls.

To determine whether the changes in lignin accumulation due to the changes in SND1 expression affect anthocyanin synthesis, we obtained seeds of the snd1ko mutant from TAIR and examined abiotic stress tolerance of this line. We then measured the anthocyanin content in the snd1ko mutant and in the SND1-overexpressing (SND1-OE) line (Fig. S1). Notably, the anthocyanin content in four-day-old seedlings was lower in the mutant and higher in the SND1-overexpressing line than in the Col-0 wild type (WT). Under normal conditions, SND1 serves as a positive regulator of lignin synthesis. Therefore, overexpression of SND1 should theoretically increase lignin accumulation and decrease anthocyanin accumulation. In contrast, lignin content is expected to decrease and anthocyanin content to increase in the mutant. A similar observation has been previously reported, whereby the overexpression of SND1 reduced lignin biosynthesis. These results indicate that SND1 is positively involved in the accumulation of anthocyanin. The expression of most flavonoid-related genes increased in the SND1-overexpressing line, but decreased in the snd1nst1 double mutant (Fig. S2). NST1 is a homologue of SND1, and marked effects on secondary wall biosynthesis are observed when both are deleted. Furthermore, we observed a decrease in the expression of PAP1 in the snd1nst1 double mutant (Fig. S2), which specifically activates the expression of genes associated with flavonoid synthesis. These results show that SND1 plays a positive role in the expression of genes associated with flavonoid biosynthesis, thereby increasing anthocyanin accumulation.

Anthocyanin is a part of the plant defence mechanism. In fact, anthocyanin often accumulates in response to stress. We verified whether SND1 has a role in plant stress responses, as the expression of SND1 is known to affect the accumulation of anthocyanin. Thus, the transcript level of SND1 was measured in Col-0 by the qRT-PCR, upon treatment with different plant hormones and stresses. In the presence of salt or mannitol, the expression of SND1 increased. Similar results were observed with abscisic acid (ABA) treatment (Fig. 1). In particular, the expression of SND1 was significantly higher under salinity stress than under other stress conditions tested. These results indicate that SND1 is related to osmotic stress, especially in response to salinity stress, as well as to secondary cell wall synthesis.
Reduced tolerance of the snd1ko mutant under salinity stress. As an extension of the above experiments, we verified whether altered anthocyanin biosynthesis due to SND1 mutation can affect abiotic stress responses in plants. As shown in Fig. 2A and B, the snd1ko mutant exhibited a very low survival rate under salinity stress, whereas that of the SND1-overexpressing line did not significantly differ from the WT. The WT showed a 20% survival rate 7 d after being transferred to the 200 mM NaCl condition, whereas the mutant showed a maximal survival rate of 5% under similar conditions. However, the difference between the SND1-overexpressing line and WT was negligible. By the seedling-transfer method, the phenotypes were confirmed under salinity stress. The snd1ko mutant exhibited a lower survival rate than the WT, whereas the survival rate of SND1-complementation (SND1-Com) line was similar to that of the WT (Fig. S3). These results indicate that the changes in SND1 expression affect the tolerance to salinity stress in plants.

To determine whether the altered sensitivity to salt stress in the snd1ko mutant resulted from the changes in the expression of ABA-responsive genes, we examined the expression of genes that were determined by qRT-PCR in eight-day-old seedlings. In the snd1ko mutant, the expression of most ABA-responsive genes increased, particularly that of NCED3, which encodes the major enzyme involved in ABA synthesis, and was induced under salinity stress (Fig. 3A). In the experiments conducted with mannitol and NaCl, the snd1ko mutant exhibited higher expression of NCED3, whereas the expression was lower in the SND1-overexpression line than in the WT (Fig. 3B). These results suggest that the expression of ABA-responsive genes is negatively regulated by SND1, which may affect the biosynthesis of ABA due to the changes in NCED3 expression.

As SND1 is known to bind directly to the Myb46 and Myb43 promoters to enhance lignin biosynthesis, we verified whether the enhanced transcript levels of ABA-responsive genes were due to these Myb genes in the snd1ko mutant. This led us to examine whether SND1 binds to the promoter of NCED3, at least in terms of salt stress response (Fig. 3A).

Enhanced ABA accumulation in the snd1ko mutant than the wild type. As NCED3 belongs to the ABA biosynthesis pathway, we determined ABA content both in eight-day-old seedlings of the snd1ko mutant and SND1-complementation (SND1-Com) line following treatment with NaCl. As observed for gene expression, the amount of ABA in the snd1ko mutant increased under both normal and salinity conditions; however, there was no significant difference between the SND1-complementation and the WT lines (Fig. 3A). These results indicate that SND1 is involved in ABA signalling, as well as in the expression of ABA-responsive genes and in the regulation of ABA accumulation.

As SND1 regulates ABA accumulation, we verified whether the snd1ko mutant exhibits altered germination rates in response to ABA. To test this, we germinated Col-0, snd1ko, and Myb46-0E snd1ko mutants in the presence of different concentrations of ABA in the medium (Fig. 4B). The snd1ko mutant showed lower germination rate in response to ABA than that in the WT. We could not revert this rate to that of WT even with the overexpression of Myb46 in the snd1ko mutant background. This indicates that SND1 is associated with ABA sensitivity during germination, as well as stress tolerance. Moreover, Myb46 is not associated with the function of SND1 in ABA response.

Direct binding of SND1 to the ABI4 promoter. As the snd1ko mutant accumulated more ABA than that of the WT, we reasoned that SND1 may control the expression of genes associated with ABA biosynthesis. This led us to examine whether SND1 binds to the promoter of NCED3 as its transcript level was enhanced in the snd1ko mutant (Fig. 5). We then performed chromatin immunoprecipitation (ChIP) to determine if SND1 can bind directly to the promoter of the NCED3 gene. Contrary to our expectation, SND1 did not bind to the
NCED3 promoter (data not shown). We further tested the promoters of other genes (ABA anabolism-related genes: ZEP, SDR1, and AAO3; and a catabolism-related gene: CYP707A3) by the ChIP assay, but found no binding activity. However, when ABA signalling-related genes, including ABI3 and ABI4, were subjected to the ChIP assay, SND1 clearly bound to the promoter of the ABI4 gene, but not to the promoter of ABI3 (Fig. 5). SND1 bound to the region encompassing −981 to −1536 bp upstream (ABI4pro #1) of the ABI4 coding DNA sequence (CDS). We further characterized the SND1 binding site in the ABI4 promoter region by dividing them into several small sections (Fig. 5A). We analyzed cis-elements of ABI4 promoter region by PlantCARE program (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/)3. By qRT-PCR, the −1386 to −1536 (q#1) and −1079 to −1179 (q#4) regions were identified to be important for SND1-ABI4 promoter interaction (Fig. 5B). To confirm this result, we conducted a traditional yeast one-hybrid assay approach in which the ABI4 promoter sequence (ABI4pro #1) was used as a bait. As shown in Fig. 5C, the SND1 protein exhibited successful binding to the ABI4 promoter, whereas empty vector did not show any interaction. Moreover, the interaction between SND1 and ABI4 promoter was confirmed via the electrophoretic mobility shift assay (EMSA). We generated the protein of the SND1 NAC domain containing 191 amino acids, and conducted EMSA with the probe of ABI4 promoter. The ABI4 promoter q#1, which includes ABA-responsive element (ABRE), was used for the EMSA. In this experiment, we also found that the SND1 NAC domain binds directly to the ABI4 promoter, especially to the part of

Figure 3. Transcript level of ABA-related genes in Col-0, snd1ko mutant, and transgenic plants in response to osmotic stress. (A) By the qRT-PCR, the relative expression of ABI1, ABI2, ABI3, ABI4, ABI5, CesA8, NCED3, NHL6, and NDR1 was determined in Col-0, snd1ko mutant, SND1-overexpressing line, and Myb46-overexpressing line/snd1ko after treatment with 200 mM NaCl for 6h. (B) The relative expression of NCED3 in Col-0, snd1ko mutant, and SND1-overexpressing line under 10 µM ABA, 300 mM NaCl, or 400 mM Mannitol for 6h. Eight-day-old seedlings were sampled for the analysis. The error bars indicate the standard error (SE) of three replicates. The asterisks represent significant differences from that of Col-0. The values with different letters were significantly different from that of WT plants (P < 0.05).
The ABA content in Col-0, snd1ko mutant, and transgenic plants. (A) The ABA content in Col-0, snd1ko mutant, and SND1-complementation line. Eight-day-old seedlings were used to extract ABA following treatment with 200 mM NaCl for 24 h. (B) Germination rate of Col-0, snd1ko mutant, and Myb46-overexpressing line/snd1ko in response to 5 or 10 μM ABA. The appearance of roots was regarded as germination 8 d after seeding on media supplemented with 5 or 10 μM ABA. The experiments included 120 seeds per sample, and the error bars indicate the standard error (SE) of three replicates. The asterisks represent significant differences from that of Col-0 (P < 0.05).

Discussion

To actively cope with environmental threats, plants have developed various physiological and molecular mechanisms. SND1 directly bound to the ABI4 promoter (Fig. 5). Moreover, the result of EMSA showed that SND1 interacts with SND1-OE plants. These results suggest that SND1 plays a role in anthocyanin accumulation, besides its role in the synthesis of lignin. However, the role of SND1 in anthocyanin biosynthesis has not been previously studied.

We investigated how the expression of SND1 varies upon various treatments with hormones under different growing conditions. The results revealed that the most significant change in SND1 was caused by salinity stress (Fig. 1). This change in the expression of SND1 in response to salinity stress may correlate with the reduced survival rate of snd1ko plants in the presence of salt at high concentration in the medium (Fig. 2). One possibility is that the snd1ko plants accumulate less anthocyanins, which serve as antioxidants. Another possibility is that SND1 functions as a transcriptional regulator of other genes, such as some of the ABA signalling genes. To evaluate this hypothesis, we screened a number of genes by the qRT-PCR to identify genes with altered transcript level in snd1ko, in response to salinity (Fig. 3A). In this analysis, the Myb46-OE/snd1ko line was included, as we wanted to know if Myb46, one of the target genes of SND1 in the lignin biosynthesis pathway, can recover the altered phenotype of snd1ko when it is overexpressed in the snd1ko background. The results revealed that Myb46 is not involved in the SND1-driven alterations in terms of ABA related stress responses (Figs 3 and 4). It has been reported that double knockout mutations of MYB46 and MYB83 result in secondary cell wall deficiency, thereby limiting plant growth. Thus, the finding of the present study imply that SND1 likely drives different sets of genes to control ABA signalling and ABA biosynthesis.

In the search for SND1 target genes, we initially focused on the NCED3 gene, based on the qRT-PCR results (Fig. 3B). The NCED genes encode 9-cis-epoxycarotenoid dioxygenase, which catalyzes the cleavage of 9-cis-epoxycarotenoid to xanthoxin in the key regulatory ABA biosynthesis step. NCED3 is important for ABA biosynthesis during drought, as evidenced by the fact that nced3 mutants exhibited increased water loss and low ABA content. Thus, the snd1ko plants may accumulate more ABA than WT, as the NCED3 transcript level was found to be higher in this mutant (Fig. 3B). In accordance with this result, we observed that the snd1ko plants accumulated more ABA than WT (Fig. 4A). The NCED1-complementation line exhibited the recovery of snd1ko in terms of ABA content. Moreover, enhanced ABA content in the snd1ko plants seems to confer increased germination sensitivity to ABA (Fig. 4B). However, by the ChIP analysis, we found that SND1 does not bind to the promoter of NCED3 (data not shown). Thus, we performed the ChIP analysis with other possible candidate genes, which revealed that the ABI4 gene is an SND1 target. As shown in Fig. 3A, the ABI4 transcript level was enhanced in the snd1ko mutant. Our detailed ChIP analysis and yeast one hybrid assay clearly demonstrated that SND1 directly bound to the ABI4 promoter (Fig. 5). Moreover, the result of EMSA showed that SND1 interacts with promoter fragment of −1386 to −1536 from ABI4 coding DNA sequence. ABI4 also has been shown to...
positively regulate seed dormancy through ABA biosynthesis by directly binding to the promoters of CYP707A1 and CYP707A2, thereby, repressing their expression. Arabidopsis CYP707A encodes ABA 8′-hydroxylase, which plays key roles in ABA catabolism, as indicated by the observation that the enhanced expression level of CYP707A1 and CYP707A2 in the abi4 ko mutant reduced ABA content. However, we did not observe any difference in the transcript levels of CYP707A1 and CYP707A2 between in WT and the snd1ko mutant (data not shown). In contrast, ABI4 is known to increase the expression of CHO1 which is an activator of NCED3. Hence, the increase in ABA content in the snd1ko mutant was not caused by the suppression of CYP707A1/2, but by the genes CHO1 and NCED3.
Abscisic acid frequently plays a central role in adaptation of plants to environmental stress\textsuperscript{27,28}. In the present study, we demonstrated that SND1 has more than one function; it can reduce ABA biosynthesis via the repression of \textit{ABI4} (Figs 3 and 5), and it can also enhance lignin biosynthesis through \textit{Myb46} activation\textsuperscript{21}. Furthermore, ABA has been shown to reprogram transcriptional schemes for improved adaptation to abiotic stress\textsuperscript{29}. In this pathway, ABA bound to \textit{PYR/PYL/RCAR} (PYRABACTIN RESISTANCE1/PYR1-LIKE/REGULATORY COMPONENTS OF ABA RECEPTORS) receptors, and inhibits \textit{ABI1}, \textit{ABI2}, \textit{HAB1}, and \textit{PP2CA} protein phosphatases, thus activating SnRK2 protein kinases\textsuperscript{18}. In addition to triggering the stress defence pathways and closing of stomata, one important function of ABA is to inhibit seedling growth. A transcriptional mechanism associated with the inhibition of the cell cycle\textsuperscript{30} and metabolism\textsuperscript{31} has been proposed in association with ABA function. Moreover, ABA modulates the function of PM H\textsuperscript{+}-ATPase\textsuperscript{32} and nutrient transporters to adjust of plant growth\textsuperscript{33}. On the basis of our results, we propose a working model of the dual function of SND1 (Fig. 6). Under normal conditions, plants can allocate all available resources to the growing points; however, they have to redirect them under growth-limiting conditions, of one kind or another. Therefore, it is plausible to reason that SND1 activates \textit{Myb46}, thereby fostering the biosynthesis of lignins to produce sufficient cell wall components needed for plant growth, while maintaining a low concentration of ABA, a growth inhibitor. Such dual function of SND1 may help plants to modulate their growth patterns to suit specific growth environmental conditions and, thus, thrive in the best possible way under such circumstances.

Methods

\textbf{Plant material and growth conditions.} The seed coat of all experimental materials was sterilized. After 3 d at 4 °C in dark, the seeds were sown on half-strength Murashige and Skoog (MS) medium supplemented with 2% sucrose (pH 5.7), as the normal growing condition. The seedlings were grown in a growth chamber at 23 °C and 60% relative humidity under long-day conditions (light 16 h, dark 8 h) for all experiments. \textit{Arabidopsis thaliana} ecotype Colombia-0 was used as the wild type (WT). The \textit{snd1} knockout mutant (SALK\_015495.54.50.x) was obtained from the Arabidopsis Biological Resource Center (ABRC).

\textbf{DNA construction and generation of transgenic plants.} Coding DNA sequences (CDS) of SND1 and \textit{Myb46} were amplified by the RT-PCR and cloned into the TOPO vector (pCR\textsuperscript{TM}8/GW/TOPO\textsuperscript{®} TA Cloning Kit, Invitrogen). The SND1-TOPO vector construct was subcloned into the pMDC32 vector\textsuperscript{34} to generate the SND1 overexpressing lines, and the pMDC83 vector\textsuperscript{34} for \textit{SND1-GFP} fusion. The \textit{Myb46-TOPO} vector construct

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**Figure 6.** Working model of SND1, negatively regulating \textit{ABI4} in the ABA biosynthesis pathway. SND1 is a master switch for the formation of secondary cell walls through the induction of \textit{Myb46} under normal conditions. For a strong and successful plant growth, SND1 directly binds to the \textit{ABI4} promoter and negatively regulates the expression of the \textit{ABI4} transcript, inhibiting the ABA biosynthesis pathway. SND1 is induced by osmotic stress and inhibits continuous ABA production under these conditions.
was subcloned into the pMDC32 vector. For SND1 complementation, a DNA construct including the sequence −1000 bp upstream to the coding DNA sequence (CDS) was cloned into the TOPO vector. The vector was subcloned into the pMDC100 vector to generate the SND1 complementation line. The subcloning constructs were transformed into Agrobacterium tumefaciens (GV3101) by electroporation. The floral dipping method was used for plant transformation. Background plant of the SND1-overexpressing line and the SND1-GFP line was Col-0. The Myb46-OE/snd1ko and SND1-complementation lines were generated in a snd1 knockout mutant background.

RNA isolation and quantitative real-time RT-PCR. The total RNA was isolated from eight-day-old seedlings following treatment with 10 µM ABA, 10 µM Indole-3-acetic acid (IAA), 10 µM Jasmonic acid (JA), 10 µM Salicylic acid (SA), 200 mM NaCl, 300 mM NaCl, 400 mM mannitol, or 200 mM sucrose for 6 h. The cDNA was synthesized using the total mRNA samples from eight-day-old seedlings and a cDNA synthesis kit (RevertAid First Strand cDNA Synthesis Kit, ThermoScientific). For quantitative real-time PCR (qRT-PCR), the cDNA was amplified using the EvaGreen MasterMix (BrightGreen qPCR MasterMix, Abm). Actin2 was used as an internal control; gene primers for the qRT-PCR are listed in Table S1.

Salinity stress phenotype analysis. The seeds were sown on filter paper placed on half-strength MS medium supplemented with 2% sucrose (pH 5.7). Two days after seeding, the filter papers with seedlings were moved onto a medium supplemented with 200 mM NaCl.

Determination of ABA content. The content of ABA was measured as previously described. The samples of eight-day-old seedlings were used to extract ABA after treatment with 200 mM NaCl for 24 h. The content of ABA was measured using the Phytodetek Elisa kit according the instructions of the manufacturer.

Analysis of cis-regulatory elements and chromatin immunoprecipitation. The cis-elements in ABI4 promoters were analyzed by PlantCARE program (http://bioinformatics.psb.ugent.be/webtools/plantcare/html)\(^\text{19}\). The chromatin immunoprecipitation (ChIP) was performed with modifications to the method originally reported\(^\text{37}\). Fourteen-day-old SND1-GFP seedlings were used to extract nuclear content with cross-link. An antibody against GFP (G1544, Sigma, https://www.sigmaaldrich.com/) was used for immunoprecipitation. The purified DNA was quantified by the qRT-PCR using the specific primers listed in Table S1.

Yeast one-hybrid assay. Full-length SND1 was fused in frame with the GAL4 activation domain of pGADT7 AD vector. The bait construct of ABI4 promoter region (ABI4pro Sac1 F: GAGCTCTAGTTTTTACTTATG TCCAAAAATATGA, and ABI4pro Kpn1 R: GGTACCTAGTAAAAGATCTAAATGCATTTTTAAT) was cloned into the pAbAi vector. The recombinant plasmid pGADT7-SND1 and ABI4 promoter bait plasmid were co-transformed into the yeast strain Y1HGold (Clontech). The yeast containing pGADT7-empty vector and ABI4 promoter bait were used as control. The transformants were cultured in SD/-Leu medium, and then transferred onto a medium supplemented with 200 mM NaCl.

Protein purification and electrophoretic mobility shift assay. To obtain recombinant protein for the electrophoretic mobility shift assay (EMSA), the DNA fragment of SND1 NAC domain was subcloned into pMAL-His-C2X vector, which is a recombinant pMAL-C2X vector (NEW ENGLAND BioLabs). The 6x-histidine (His) tag was fused in-frame with the C-terminus of maltose-binding protein (MBP) of pMAL-His-C2X vector. The primer for SND1 NAC domain cloning is listed in Table S1. The construct obtained was transformed into E. coli strain BL21 (DE3). To express the recombinant protein, the recombinant E. coli was grown in LB liquid medium at 37°C until the OD (600) reached 0.7, and then 0.1 mM isopropyl-b-D-thiogalactopyranoside was added to the medium and the cells were grown again at 18°C for 18 h. After centrifugation at 3500 rpm for 1 h, the supernatant was removed and the bacteria were stored at −20°C until the isolation of protein. The Ni-His tag column was used to purify the recombinant protein. The ABI4 promoter fragment was amplified by the PCR with both 5' and 3' biotin-labelled primers. The primer sequences for the ABI4 promoter fragment were similar to those of the ABI4pro q1 primer for the ChIP assay. The EMSA assay was conducted according to the instructions provided in the LightShift\(^\text{®}\) Chemiluminescent EMSA kit insert (Thermo Scientific).

Germination rate analysis. The seeds were sown on half-strength MS medium supplemented with 2% sucrose (pH 5.7) or ABA-supplemented medium. Eight days after seeding, the roots were counted to analyse germination rates. One-hundred seeds were used for the analysis and three independent experiments were conducted.

Statistical analyses. Each experiment was replicated at least thrice. The statistical analyses were performed by the one-way ANOVA, followed by Tukey’s test for comparison of means at 95% confidence level.

Gene accession number. The accession numbers of the genes used in this study are as follows: ABF1 (AT1G49720), ABF2 (AT1G45249), ABF3 (AT4G34000), ABF4 (AT3G19290), ABI1 (AT4G26080), ABI2 (AT5G57050), ABI3 (AT3G24650), ABI4 (AT2G40220), ABI5 (AT2G36270), CesA8 (AT4G18780), COR15a (AT2G42540), CHI (AT3G55120), CHS (AT5G13930), DFR (AT5G42800), F3H (AT3G51240), F3’H (AT3G51420), FLS1 (AT5G08640), LDOX (AT4G22880), Myb46 (AT5G12870), NCD3 (AT3G14440), NDR1 (AT3G26000), NHL6 (AT1G65690), NIT1 (AT2G46770), PAP1 (AT1G56650), RD29a (AT5G52310), RD29b (AT5G23000), SND1 (AT1G32770).
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Author Contributions

Chan Young Jeong: qRT-PCR and ChiP assay, EMSA, Yeast one-hybrid assay, and MS writing. Won Je Lee: Anthocyanin detection. Hai An Truong and Cao Son Trinh: Complementation assay. Joo Yeon Jin: Abiotic stress test. Sulhee Kim and Kwang Yeon Hwang: Protein purification. Chon-Sik Kang: ABA extraction. Joon-Kwan Moon: Sodium contents determination. Suk-Whan Hong and Hojoung Lee: Experiment designs and MS writing.

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