Arabidopsis YL1/BPG2 Is Involved in Seedling Shoot Response to Salt Stress through ABI4

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The chloroplast-localized proteins play roles in plant salt stress response, but their mechanisms remain largely unknown. In this study, we screened a yellow leaf mutant, yl1-1, whose shoots exhibited hypersensitivity to salt stress. We mapped YL1 to AT3G57180, which encodes a YqeH-type GTPase. YL1, as a chloroplast stroma-localized protein, could be markedly reduced by high salinity. Upon exposure to high salinity, seedling shoots of yl1-1 and yl1-2 accumulated significantly higher levels of Na⁺ than wild type. Expression analysis of factors involved in salt plant stress response showed that the expression of ABI4 was increased and HKT1 was evidently suppressed in mutant shoots compared with the wild type under normal growth conditions. Moreover, salinity effects on ABI4 and HKT1 were clearly weakened in the mutant shoots, suggesting that the loss of YL1 function impairs ABI4 and HKT1 expression. Notably, the shoots of yl1-2 abi4 double mutant exhibited stronger resistance to salt stress and accumulated less Na⁺ levels after salt treatment compared with the yl1-2 single mutant, suggesting the salt-sensitive phenotype of yl1-2 seedlings could be rescued via loss of ABI4 function. These results reveal that YL1 is involved in the salt stress response of seedling shoots through ABI4.

High salinity is a serious factor that influences plant productivity. It affects various aspects of plant physiology and metabolism by inducing osmotic stress and ion toxicity. The early-occurring osmotic stress triggers physiological changes, such as membrane interruption in roots and reduction of water absorption capacity in plants. Ion over-accumulation, which is the second phase of salt stress, can induce severe Na⁺/K⁺ imbalance and toxic effects.

Plants have evolved different molecular mechanisms to adapt to hyperionic stress. The calcium-responsive salt overly sensitive (SOS) regulatory pathway, which is mainly for ion homeostasis, has been established in Arabidopsis. SOS3, a myristoylated calcium-binding protein, recognizes salt-elicited cytosolic calcium signals and then interacts with and activates SOS2 for signal transmission. SOS1, a plasma membrane Na⁺/H⁺ antiporter and regulated by SOS3 and SOS2, aids in sodium transport from root cells back into the soil or from epidermal cells into the xylem. Overexpression of SOS1 confers plant salt stress resistance. High-affinity K⁺ transporter (HKT1), an important regulator that is independent of the SOS pathway and exhibits significant functions in root and shoot ion homeostasis, has been extensively studied. HKT1, as a K⁺/Na⁺ symporter, is highly expressed in the root stele and leaf vasculature and retrieves sodium from the root-to-shoot xylem sap in Arabidopsis. AtHKT1 knock-out mutant leaves exhibit high sensitivity to salt stress because of excessive sodium accumulation, and AtHKT1 overexpression in roots enhances the salt tolerance of the entire plant. In addition, the tonoplast-localized Na⁺/K⁺/H⁺ exchanger NHX1 confers Na⁺ or K⁺ storage into vacuoles. AtNHX1 overexpression could reduce Na⁺ stress through enhancing intracellular K⁺/Na⁺ ratios in tomato.

The phytohormone abscisic acid (ABA) exerts a significant function for coping with salt stress. The ABA-deficient mutants aba1, aba2, and aba3 show a readily wilting phenotype under salt or drought stress. ABSCISIC ACID INSENSITIVE (ABI) 4 was first isolated from a screen for ABA-insensitive mutants during seed germination. ABI4, as a member of the plant-specific AP2/EREBP family, is involved in many signal transduction pathways, such as sugar signaling and mitochondrial/chloroplast retrograde signaling. The abi4

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mutant exhibits salt stress resistance because less sodium is accumulated in plant shoots. ABI4-overexpressing (dexamethasone-induced) plants show increased salt sensitivity because ABI4 downregulates HKT1 expression by directly binding to the promoter ABE-element GC(C/G)GCTT(T)23.

It is generally accepted that high salinity can cause photosynthesis inhibition in plants, and leaf growth is very sensitive to salt stress. This phenomenon may be attributed to the disruption of chloroplast development24,25. CO2 fixation is sensitive to environmental stresses. Therefore, salt stress can inhibit the repair of PS II via the ROS-induced suppression of PS II protein synthesis, which in turn triggers an imbalance between the photo-damage and repair rates of PS II26,27. Moreover, recent studies have suggested that the chloroplast proteins also play roles in plant salt stress response28–30. However, the mechanisms are largely unclear. In this study, we screened the yellow leaf 1-1 (yl1-1) mutant. The shoots of yl1-1 showed evident salt stress-sensitive phenotypes. We demonstrated that YL1, as a chloroplast protein, is involved in the high salinity response of seedling shoots through ABI4.

Results
Phenotypes of yl1-1 Mutant. Arabidopsis seedling shoots usually exhibit pale coloration and stunted phenotypes under salt stress conditions (Fig. 1a). We are interested in mutants that the seedling shoots exhibit extremely sensitive phenotypes under salt stress. The mutant yl1-1 was isolated from approximately 30,000 ethane

![Figure 1. Salt stress sensitive phenotypes of yl1-1 seedling shoots. (a) Phenotypes of seedlings under control or salt stress conditions. Each lane represents the corresponding seedling grown for 5 days after germination under the indicated condition (NaCl, 150 mM). Three independent experiments were performed with similar results and one representative is showed. Scale bars = 0.5 mm. (b) Phenotypes of wild type or yl1-1 plants grown in different salinity stresses (NaCl, NaNO3, KCl, KNO3, and control; 150 mM) for 5 days after germination. Three independent experiments were performed with similar results and one representative is showed. Scale bar = 5 mm. (c) The percentages of the fully expanded seedling cotyledons showed in (b). Values are means ± SE of three independent replicates.](image)
after germination (Fig. 2b). In addition, the Fv/Fm and endogenous contents of chlorophyll in the mature plants of type and treatment, the cited evident pale-green leaf phenotypes under normal growth conditions (Figs 2b and S2a). Under salt stress in salk_068713), was obtained from ABRC stock (Figs 2b and S2a). Western blot, gene full-length amplification PCR plant (YL1)(an Arabidopsis wild ecotype) and self-fertilization to generate the F2 population. We mapped YL1 to At3g57180 (Brassinazole Insensitive Pale Green 2, BPG2), positioned it between the BAC markers F24I3 and F28O9, and identified a G to A base substitution, which caused a D to N change in position 574 of the amino acid sequence (YL1), which suggested a knock-out mutant (Fig. 2c,d). The yl1-1com, yl1-2, and yl1-1 complementary plant yl1-1com. Primers qp1 and qp2 showed in a were used. The internal control gene was Actin2.

Figure 2. Positional cloning of yl1-1. (a) Map position of yl1-1 on chromosome 3. Some of the used BAC markers are indicated. Gene structure of At3g57180, BPG2 is shown. The yl1-1 mutated site of G to A (amino acid of D to N) and the inserted position of a YL1 T-DNA insertion mutant, yl1-2, are all indicated. Short arrows represent primers used in (c,d). (b) Phenotypes of wild type, yl1-1, yl1-2, yl1-1com, and yl1-1 yl1-2 (cross F1 line) seedling grown under control or 150 mM NaCl condition for 5 days after germination. (c) qRT-PCR analysis of yl1-1 expression levels in wild type, yl1-1, yl1-2, and yl1-1 complementary plant yl1-1com. Primers qp1 and qp2 showed in a were used. The internal control gene was Actin2.

methylsulfonate (EMS)-mutagenized Col-0 M2 seedlings, which conferred a pale-green shoot phenotype under normal growth conditions (Figs 1a and S1). However, under salt stress conditions, shoot of yl1-1 showed evidently stunted phenotype compared with wild type (Figs 1a and S1), while little differences in root development could be observed (Fig. S1). Three additional salts (NaNO3, KCl, or KNO3) were used in seedling growth experiments to understand the phenotypes of yl1-1 hypersensitivity to salt stress better. The results showed that the percentages of the fully expanded cotyledons of yl1-1 seedlings were significantly lower in growth conditions with NaCl or NaNO3 than with KCl or KNO3 (Fig. 1b,c). By contrast, wild type seedlings did not exhibit clear differences under these different salt treatments (Fig. 1b,c). These observations suggest that Na+ toxicity leads to stunted yl1-1 shoot phenotypes.

Positional Cloning of yl1-1. A map-based cloning approach was applied to identify the target gene(s) responsible for the yl1-1 phenotypes (Fig. 2a). F1 plants were generated by crossing yl1-1 with Landsberg (an Arabidopsis wild ecotype) and self-fertilization to generate the F2 population. We mapped YL1 to At3g57180 (Brassinazole Insensitive Pale Green 2, BPG2), positioned it between the BAC markers F24I3 and F28O9, and identified a G to A base substitution, which caused a D to N change in position 574 of the amino acid sequence (Fig. 2a).

Previous studies reported that BPG2, as a YqeH-type GTPase, participates in chloroplast rRNA maturation. To determine whether the lack of YL1 is responsible for the phenotypes of yl1-1, we introduced the full-length open reading frame of YL1 to the yl1-1 mutant under the control of the 35S promoter. The complemented plant (yl1-1com) showed a normal leaf phenotype (Figs 2b and S2a). A mutant of At3g57180, yl1-2 (bpg2-2, salk_068713), was obtained from ABRC stock (Figs 2b and S2a). Western blot, gene full-length amplification PCR from cDNA, and real-time quantitative PCR (qRT-PCR) analyses showed that YL1 expression was extremely low in yl1-2, which suggested a knock-out mutant (Fig. 2c,d). The yl1-1, yl1-2, and cross F1 line (yl1-1 yl1-2) exhibited evident pale-green leaf phenotypes under normal growth conditions (Figs 2b and S2a). Under salt stress treatment, the yl1-1, yl1-2, and cross F1 line showed extremely stunted cotyledon phenotypes, whereas the wild type and yl1-1com plants exhibited normal phenotypes except for the decrease of chlorophyll contents at day 5 after germination (Fig. 2b). In addition, the Fv/Fm and endogenous contents of chlorophyll in the mature plants of yl1-1, yl1-2, and cross F1 line were significantly lower than those in the wild type and yl1-1com (Fig. S2b,c). These results indicate that the yl1-1 phenotypes are caused by YL1 function loss.

Subcellular Localization and Expression Pattern of YL1. Previous studies stated that YL1 mainly localizes in chloroplast. To further determine details of YL1 subcellular localization, we constructed a YL1-GSG-GFP plasmid containing a flexible peptide (GGGSSSSGGG) between the YL1 and GFP gene sequences (Fig. 3a). GFP fluorescence was clearly detected in the chloroplasts of YL1-GSG-GFP transgenic plant protoplasts (Fig. 3b).
Immunoblot analysis of the stroma and membrane fractions from Percoll-purified chloroplasts demonstrated that YL1 protein is localized in the stroma, and not in the membrane (Fig. 3c). Here, the chloroplast stroma protein RbcL and thylakoid membrane protein D1 were used as control (Fig. 3c). In addition, the localization of the mutated YL1 (mYL1) in yl1-1 is not changed (Fig. 3b), further confirming that the mutation elicits protein function defects.

The expression pattern analysis used in β-glucuronidase (GUS) staining showed that the YL1 gene is expressed in most of the green tissues throughout the plant growth cycle, even in germinating seeds (Fig. 3d). However, the GUS activity is extremely low in roots and mature seeds (Fig. 3d), suggesting low expression of YL1 in these tissues. To detect the effect of salt stress on the expression of YL1, total RNA was isolated from 5-d-old wild type seedling shoots harvested after salt treatment. The results of qRT-PCR showed that the levels of YL1 transcripts were evidently reduced with increasing salt concentration or treatment time, which were further confirmed by immunoblot and GUS activity analysis (Figs 3e,f and S3). When plants were treated with 150 mM NaCl for 12 h, YL1 expression decreased to less than half of that of the non-treated plants (Fig. 3e,f). In addition, no matter treated with or without NaCl, YL1 transcripts were hardly detected in roots (Fig. S4). These results indicate that the YL1 could be significantly downregulated by salt stress in seedling shoots.

**Loss of YL1 Function Causes Shoot Na⁺ Accumulation under Salt Stress.** Basing on the results presented in Fig. 1b, we speculate that Na⁺ homeostasis is disrupted in yl1-1 shoot tissues under salt stress conditions. Thus, Na⁺ and K⁺ levels were measured using atomic absorption spectrophotometry. The Na⁺ contents
in the shoots were substantially higher in yl1-1 and yl1-2 than in wild type plants treated with 150 mM NaCl for 2 days (Fig. 4a). By contrast, no differences in Na⁺ contents were observed in roots among these genotypes (Fig. 4b). K⁺ levels were similar among the three genotypes regardless of tissue type (Fig. 4c,d). Thus, we conclude that Na⁺ is over-accumulated in the shoots of yl1 seedlings under salt stress.

Loss of YL1 Function Affects ABI4 Expression in Seeding Shoots. To identify the factor(s) that possibly play roles in Na⁺ over-accumulation in the shoots of yl1-1 and yl1-2, transcripts of several genes involved in plant salt stress response were detected. The results showed that, regardless of treatment with or without salt, no clear differences were observed in the expression levels of the SOS pathway genes, ABA biosynthesis pathway genes, or NHX1 among the studied genotypes (Figs S5 and S6). Similar results of ABI4 or HKT1 transcription levels were found in the roots of the detected genotypes (Fig. 5a,b). However, in the shoots of the mutant seedlings, ABI4 expression was three- to fourfold higher, whereas HKT1 transcription levels were substantially lower than those in the wild type grown under normal growth conditions (Fig. 5c,d). Moreover, after 12 h of salt stress treatment, evident inductions of ABI4 and reductions in HKT1 transcripts were observed in the shoots of wild type and roots of all detected genotypes (Fig. 5), but no significant changes of ABI4 or HKT1 could be observed in the mutant shoots (Fig. 5c). These results suggest that loss of YL1 function impairs ABI4 and HKT1 expression in seeding shoots. A previous study stated that ABI4-overexpressed plants are sensitive to salt stress because of HKT1 depression23. Based on these evidences, the salt-sensitive phenotype of the yl1 mutant shoot is possibly tightly associated with the high levels of ABI4.

ABI4 Functions in Salt Stress Response of yl1 Mutant Seedlings. To further confirm the above issue, a double mutant, yl1-2 abi4, was created. The 10-d-old seedling shoot of yl1-2 abi4 showed a pale-green phenotype due to the low chlorophyll contents, revealing that the YL1 roles in leaf coloration are independent of ABI4 (Fig. 6a,b). YL1 transcripts were not clearly influenced by the abi4 mutant, indicating that YL1 expression was also ABI4-independent (Fig. 6c). However, HKT1 mRNA levels were significantly higher in the shoots of yl1-2 abi4 than in the yl1-2 single mutant, which was similar to that in abi4 (Fig. 6d). Furthermore, under high-salinity growth conditions, the percentages of the fully expanded cotyledons of yl1-2 abi4 seedlings were similar to those of the wild type and abi4 (Fig. 6e,f). The seedling shoots of salt-treated yl1-2 abi4 also accumulated lower levels of Na⁺ than the yl1-2 single mutant, whereas K⁺ contents did not considerably change in all detected genotypes (Fig. 6g–j). Thus far, the abovementioned results clearly indicate that the salt stress-sensitive phenotype of yl1-2 shoot could be completely rescued by the loss of ABI4 function.
Discussion

Hyperionic stress causes plant growth inhibition or plant death by disturbing the physiological functions of the shoots and roots. This study focused on shoot phenotypes to elucidate the mechanisms of plant response to salt stress. *yl1-1*, a mutant with severely stunted shoot phenotype under salt stress conditions, was isolated from an EMS-mutagenized library in Col-0 background (Fig. 1a). YL1, which is mainly localized in the chloroplast stroma (Fig. 3b,c), is a YqeH-type GTPase. GTPase, including Obg, Era, YlqF, YqhC, YsxC, YqeH, and YloQ-type families, exists in almost all organisms and is involved in regulating diverse cellular processes. However, the YqeH is only conserved in bacteria and plant chloroplast. In *Bacillus subtilis*, YqeH is involved in 30S ribosome subunit biogenesis and 16S rRNA maturation. In plants, BPG2 (YL1) plays roles in chloroplast ribosome RNA maturation. Kim *et al.* proposed that BPG2 (YL1) binds to plastid RNA for chloroplast translation apparatus assembly. AtNOA1/RIF1, a YqeH-type GTPase homolog in *Arabidopsis*, confers an atypical nitric oxide synthase activity, plastid ribosome function, and root growth behavior control. OsNOA1 RNAi rice seedlings show chlorotic phenotype. Nevertheless, no study has investigated the involvement of YqeH-type proteins in environmental stress response.

*Na*⁺ accumulation was high in the shoots of *yl1-1* and *yl1-2* under salt stress conditions (Fig. 4a), suggesting that the mechanisms of Na⁺ exclusion were interrupted because of the loss of YL1 functions. Na⁺ exclusion broadly refers to two mechanisms: Na⁺ efflux from root epidermis and decrease of Na⁺ delivery from root to shoot. SOS pathway genes play vital roles in Na⁺ efflux in roots. Homologs of SOS1 from rice, wheat, and tomato have been characterized in controlling Na⁺ transport. YL1 was difficult to detect in roots, and the expression levels of SOS pathway genes were not altered in the *yl1* mutants (Figs 3a and S6). Moreover, loss of YL1 functions did not clearly affect root development or ion homeostasis under controlled or high salinity conditions (Figs 4 and S1). These results suggest that the salt hypersensitivity of mutant shoots is not caused by the reduced Na⁺ efflux from root epidermis but probably by the increase of Na⁺ delivery from root to shoot. To our knowledge, Na⁺ delivery from root to shoot mainly depends on the transpiration stream in the xylem. HKT1 could...
directly retrieve Na\(^+\) from the xylem sap back to the phloem of the shoot and unload it in the root, which in turn reduces Na\(^+\) accumulation in the shoot\(^{13,15}\). In wheat, TmHKT1;4-A2 is expressed in leaf sheath and reduces Na\(^+\) concentration in the shoot\(^{48}\). ABI4 has been recently reported to act as a negative regulator that could directly bind to the promoter region and inhibit HKT1 expression in Arabidopsis\(^{23}\). In the shoots of yl1 mutants, ABI4 was highly expressed, and the HKT1 transcription levels were approximately half of those of the wild type (Fig. 5). By contrast, in the shoots of the yl1-2 abi4 double mutant, the transcripts of HKT1 were evidently higher than...
those in yl1-2 (Fig. 6d). Furthermore, the loss of ABI4 function could rescue yl1-2 shoot salt-sensitive phenotypes (Fig. 6e,f). These observations suggest that HKT1 is depressed by high levels of ABI4 in shoots of yl1 mutants.

ABI4, which binds to the coupling element 1 (CE1, CACCG) in vitro, is involved in regulating a large number of genes. ABI4 plays key roles in sugar, ABA, and pathogen-response signaling pathways through binding to S-box or G-box sequences. Furthermore, ABI4 expression could be regulated by many factors. During seed maturation, germination, and the early-stage of seedling development, ABI4 could be induced by ABA and cytokinin and be repressed by auxin. ABI4 could also directly bind to its own promoter CE1-like element and activate gene expression. Previous studies also reported that ABI4 is significantly downregulated in the wrky18 and wrky40 mutant seedlings. Moreover, the transcription factor SCR was also proven to directly bind to ABI4 promoter and negatively regulate ABI4 expression.

At the seedling stage, similar to YL1, ABI4 expression is readily detected in shoots. Our results showed that ABI4 was more highly expressed in the shoots of yl1 mutants compared with in the shoots of the wild type (Fig. 5c), whereas in the roots of yl1-1 or yl1-2, ABI4 expression was almost unchanged (Fig. 5a). Consistent with previous study, ABI4 transcripts could be clearly increased by salt stress signaling in the wild type (Fig. 5c), which is contrary to YL1 (Fig. 3e,f). However, in shoots of yl1, the salinity effect on ABI4 expression was clearly weakened (Fig. 5c). The abovementioned results suggest that YL1 may be a potential factor that acts upstream of ABI4 in salt stress signaling because the YL1 expression is independent of ABI4 (Fig. 6c). Together with the phenotype of yl1-2 abi4 under salt stress treatment (Fig. 6e), we reveal that ABI4 plays roles in seedling shoot salt-sensitive phenotype of yl1 mutants. Considering that the YL1 protein localizes in the chloroplast and ABI4 is a nucleus-localized transcription factor, several signals may be derived from the chloroplast and effectively transmitted to the nucleus when YL1 dysfunctions. Several studies stated that ABI4 plays key roles in chloroplast retrograde signaling. ABI4, which could bind the Lhcb family gene promoter CCAC-motif, acts downstream of GUN1 and PTM and negatively regulates target gene transcriptions. Thus, whether YL1 regulates ABI4 through retrograde signaling need to be identified in future studies.

Recent studies have identified several genes are involved in plant shoot response to salt stress. Nevertheless, the regulatory mechanisms of plant shoot salt tolerance remain unclear. yl1-1 was investigated, and the results suggested that chloroplast proteins such as YL1 could be involved in plant salt stress response through nuclear stress-responsive factors. We speculate that the reduction of YL1 and induction of ABI4 under high salinity conditions may be an adaptive mechanism to achieve Na\(^+\) equilibrium in the entire plant, which needs to be investigated further. Although the signaling pathway is unclear, our results may open a new insight into the association of plant salt stress response and the chloroplast.

Methods

Plant Materials and Growth Condition. All wild type plants in the study are Arabidopsis thaliana Col-0. The mutants, yl1-1, yl1-2 (salk_068713), and abi4 (salk_080095) are in the Col-0 genetic background. NaClO (0.1%) and ethanol (70%) are used for seed sterilization. Seedlings are grown in solid 1/2 Murashige and Skoog (MS) medium containing 1% sucrose.

Constructs and plant transformation. For construct used in yl1-1, CDS sequence of YL1 was subcloned into pBI121 vector with recognition sites for the restriction enzymes BamHI and SalI. For YL1-GSG-GFP (mYL1-GSG-GFP) construct, a flexible peptide GSG (GGGSSSSGGG) was added between YL1 (mYL1) CDS sequence of termination coden free and GFP sequence. The YL1-GSG-GFP (mYL1-GSG-GFP) sequence was also cloned into pBI121 vector. Constructs were introduced into Agrobacterium tumefaciens GV3101 for plant transformation. Transgenic plants were selected on 1/2 MS medium containing 50 mg L\(^{-1}\) kanamycin. Plants were selfed twice and T3 homozygous plants were used. Primers are listed in Table S1.

Stress Treatment and Phenotypes Recording. Surface sterilized seeds were plated in solid 1/2 MS medium described above containing 150 mM NaCl or not. Plated seeds were 4°C-dark treated for 2 d, then transferred to incubator (22°C, 16/8 h light/dark) and grown for 5 d. The number of plants with (n) or without (m) fully expanded cotyledons was recorded. Then the percentages of fully expanded seedling cotyledons were calculated with (n/n + m) *100%.

Transcript Analysis. For gene transcription analysis under salt conditions, 4-d-old seedlings grown under normal conditions were transferred to the MS medium containing 0, 100, or 150 mM NaCl and then treated for different times (2–36 h). The shoots and roots were separately harvested and total RNA was extracted with Trizol (invitrogen). cDNA synthesis was performed using PrimeScript reverse transcriptase (RT) with oligo dT primer (invitrogen). PCR system (Bio-Rad, C1000) using SYBR green real-time PCR master mix (Takara). Detection for each gene was performed by an CFX96 real-time PCR system (Bio-Rad, C1000) using SYBR green real-time PCR master mix (Takara). Detection for each gene transcript was performed for at least three biology replicates and each bio-replicate containing three technical replicates. All used primers are listed in Table S1.

YL1 Promoter Construction and GUS staining. The pYL1-GUS was constructed by subcloning a 1.5 kb fragment upstream of the YL1 translation start site into pBI122 binary vector. GUS staining analysis for different tissues and GUS activity analysis in seedlings for different treatment were according to Yan. Fluorescence of GUS was measured with a Microplate Spectrofluorometer (IC Measurement Acc for FL Solutions, F-4500, HITACHI). Primers are listed in Table S1.

Immunoblot Analysis. Plant materials were prepared according to the methods described in Transcript analysis. Total proteins of such as seedling shoots were extracted using Plant Protein Extraction Kit (CWIBIO) and measured using a nano-drop instrument (Nano-Drop, ND-1000 Spectrofluorometer). The polyclonal antibody of...
YL1 was obtained from rabbits by Abmart (China). The RbcL, D1 and Actin antibodies were all purchased from Agrisera. Proteins after electrophoresis were blotted to nitrocellulose membranes and then probed with specific antibodies. The nitrocellulose membranes were visualized with an enhanced Lumi-Light Western Blotting Substrate kit (Thermo Scientific).

**Ion Content Measurement.** For measurement to Na⁺ and K⁺, the 3-d-old seedlings grown under normal conditions were transferred to the MS medium containing 0 or 150 mM NaCl and then grown for 2 d. The shoots from these 5-d-old plant were separately harvested, dried for 48 h at 80 °C and then ground to powder. The same mass tissue powder was digested in concentrated (69%, v/v) HNO₃, for 24 h at room temperature for elemental extraction. Na⁺ and K⁺ concentrations was determined by atomic absorption spectrophotometry (novAA300, analytikjena).

**Chloroplast Isolation.** 5-d-old seedling cotyledons were harvested and grounded in isolation buffer (20 mM HEPES/KOH, pH 8.0, 0.3 M sorbitol, 5 mM MgCl₂, 5 mM EGTA, 5 mM EDTA, and 10 mM NaHCO₃). The homogenate was filtered and centrifuged at 3000 g for 3 min. The pellet was resuspended in 1 mL isolation buffer. The intact chloroplasts are isolated through Percoll gradient method⁶⁰. Thylakoid membranes and stroma proteins were prepared from isolated intact chloroplasts.

**Chlorophyll content measurement.** Cotyledons or leaves of plant were collected, fresh weighed and washed in distilled water. Chlorophyll was extracted in 80% (v/v) acetone at 25 °C in darkness for 24 h, and concentration of chlorophyll a/b was determined according to Komatsu³¹.

**Accession Numbers.** Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL data libraries under the following accession numbers: YL1, At3g57180; ABI4, At2g40220; HKTI, At1g10310; SOST, At2g18980; SO5, At5g5410; SO5, At5g24270; NHX1, At5g27150; ABI1, At5g67030; AB2, At1g52340; AB3, At1g16540; AA03, At2g27150; NCED3, At5g14440.

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Author Contributions
P.-C.L., J.-G.H., C.-A.W. and C.-C.Z. designed the study. P.-C.L., S.-W.Y., Y.-Y.L. and P.S. performed the research. P.-C.L. and J.-G.H. analyzed the data. P.-C.L. and C.-C.Z. contributed to writing the article. All authors reviewed the manuscript.

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