The regulation of salt and drought stress responses by SDR and its interacting proteins in arabidopsis

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ABSTRACT

The ubiquitin/26S proteasome pathway is key to protein degradation in plants. Its specificity often orchestrated by ubiquitin-protein ligases (or E3s), which facilitate the translocation of ubiquitin to appropriate targets. F-box protein is one of the subunit of E3 ligases SCF (Skp1-Cullin/CDC53-F-box). It has been reported that F-box protein is not only related to plant growth but also abiotic stress. In this study, the protein was found localised in the nucleus and its function was identified. It demonstrated that on salt treatment SDR is involved in salt and drought stress response in Arabidopsis. However, the function of most F-box proteins is unknown. In this paper, the full length of the F-box protein SDR gene was cloned by traditional reverse molecular biology methods, and related transgenic materials were constructed. Bioinformatics analysis of the cis-element of the promoter of F-box protein was used to screen F-box proteins that may be stressed by plants. We found a large number of abiotic stress response elements such as drought stress response elements, salt stress response elements, and heat shock response elements in the promoter sequence upstream of the SDR (At5g15710) gene. The results show that SDR can be induced by ABA, heat shock, and salt, but expression is suppressed under drought treatment.

KEYWORDS: SDR, Genes, F-Box, Ubiquitin/26S

INTRODUCTION

Salt stress is a major abiotic factor leading to loss of agricultural productivity worldwide (Zhan et al., 2019). Plant survival under abiotic constraints depends on the perception of environmental signals that lead to signal transduction pathways that in turn alter gene expression in order to put in place protective mechanisms. Salinity and osmotic stress are huge constraints amongst the abiotic stresses and possess a couple of deleterious effect on plant development lowering productivity and hampering agriculture across the globe (Yadav et al., 2020). Currently, the main objective in plant breeding is increasing tolerance to environmental stress. However, mechanisms that control tolerance, such as tolerance to water deficit are complex and involves several genes. (Marques et al., 2017).

In saline and dry environment, plant water uptake is reduced due to low osmotic potential of the soil. To counter the water dehydration, plants have employed various mechanisms such as; stomatal closure, regulation of water fluxes and biosynthesis of osmo-protectants such as salt stress. Inadequate water has been found to negatively influence growth in plants. To overcome such constraints plants have evolved various mechanisms of conserving this scanty valuable resource. One way plants have improvised in saline environment is regulation and maintenance of ionic concentration at a certain threshold (Julkowska & Testerink, 2015; Yadav et al., 2020).

Plants subjected to stress seek to adapt to the stress by expressing specific genes. Genes responsive to water deficit are largely regulated by abscisic acid (ABA). However, there are other response genes that are not regulated by ABA, indicating the existence of several regulatory molecular mechanisms (Marques et al., 2018). ABA has been found to have a core roles in plant drought stress responses (Cutler et al., 2010). Upon drought treatment, ABA content quickly elevates leading to the formation of ternary complexes of ABA, PYRABACTIN RESISTANCE1-LIKE/ REGULATORY COMPONENTS OF ABA RECEPTORS ABA receptors of the START protein family, and type 2C protein phosphatase (PP2C) proteins according to Brandt et al. (2012) freeing Snf1-Related Protein Kinase1 from the inhibition of PP2C protein (Melcher et al., 2009). The activated Snf1-Related Protein Kinase1 phosphorylates downstream ion channels and a couple of transcription factors that binds the ABA response element and leading to regulation of the expression of ABA-responsive genes Fujita et al. (2013).
leading to maintenance of water in plant cells under water-deficit conditions. In addition, other dehydration proteins accumulate for plant protection during stress conditions (Hauser et al., 2011).

F-box proteins, which are part of SCF (for S-Phase Kinase-Associated Protein1/Cullin or Cell Division Cycle53/F-box protein) E3 ubiquitin ligases have been shown to play vital functions during growth and development in plants. Arabidopsis genome encodes more than 700 putative of these proteins (Gagne et al., 2002). However, a few of the proteins are reported to be involved in abiotic stress responses such as ABA and drought response pathways (Bu et al., 2014; Hwang et al., 2020).

Drought Tolerance RepressoR1 (DOR1) is an F-box protein expressed in guard cells (Zhang et al., 2008). Consistent with the drought tolerant phenotype, the dor1 mutant is hypersensitive to ABA-induced stomatal closure and has higher ABA content. But dor1 did not show any ABA-related phenotypes during seed germination and the early seedling development stage in a research study by Zhang et al. (2008).

The Empfindlicher im Dunkelroten Licht1-Like Protein3 is another ABA-induced F-box protein that has got the ability of interacting with multiple Arabidopsis S-Phase Kinase-Associated Protein1-like proteins with differential strength. Biological assays showed that Empfindlicher im Dunkelroten Licht1-Like Protein3 positively regulates ABA inhibition of seed germination, early seedling development, and root growth (Koops et al., 2011; Wang et al., 2010).

MAX2 (MORE AXILLARY GROWTH2), another F-box protein, has been previously shown to function in strigolactone-mediated regulation of branching, karrinin signaling, temperature signaling, and senescence pathways (Brewer et al., 2015). It also positively regulates photomorphogenesis under all three (blue, red, and far-red) light conditions (Nelson et al., 2011). Although all the max mutants (max1, max2, max3, and max4) share an increased shoot-branching phenotype, only MAX2 but not the other MAX proteins (MAX1, MAX3, and MAX4) involved in strigolactone biosynthesis can affect plant photomorphogenesis. In addition, only max2 displayed defects in karrinin signaling implying that MAX2 has diverse roles in other pathways not regulated by strigolactone (Bu et al., 2014).

Moreover, studies have found that LCR (ABA-induced F-box protein) not only plays an important role in regulating plant growth and development, but also participate in regulating plant stress response. The results indicate that LCR regulates plant responses to salt and drought stress by relying on ABA (Song et al., 2016). In addition, the F-box protein More Axillary Growth2 (MAX2) is involved in regulating plant drought stress response. MAX2 appears to be very sensitive to drought, however, overexpressing MAX2 plants did not show related traits. This shows that MAX2 is not a limiting factor for regulating abiotic stress, and may need to co-regulate the stress response of plants with other factors (Bu et al., 2014).

The ubiquitin / 26S proteasome pathway is involved in all aspects of plant growth and development. F-box protein is a subunit of the E3 ubiquitin ligase SCF complex and determines the specificity of the substrate. It plays an important role in the process of ubiquitin degradation of substrate proteins. Recently, F-box protein has been found to be associated with plant growth, development and stress (Maldonado-Calderó et al., 2012; Shu et al., 2017).

Despite the function of most F-box proteins is being unknown in this paper, the full length of the F-box protein SDR gene was cloned by traditional reverse molecular biology methods, and related transgenic materials were constructed. Bioinformatics analysis of the cis-element of the promoter of F-box protein was used to screen F-box proteins that may be stressed by plants. We found a large number of abiotic stress response elements such as drought stress response elements, salt stress response elements, and heat shock response elements in the promoter sequence upstream of the SDR (At5g15710) gene. The functions of SDR under salt and drought stress were identified. The results show that SDR can be induced by ABA, heat shock, and salt, but its expression is less under drought treatment. In addition, SDR is expressed in all stages of the plant, and its expression is relatively high in rosette leaves and flower organs.

**MATERIALS AND METHODS**

*Arabidopsis thaliana.* L. Columbia ecotype Col-0, purchased from Arabidopsis Biological Resource Center (ABRC; Ohio State University, Columbus, OH). The experimental plant transgenic materials were set in Arabidopsis Colombia ecotype Col-0 as background. Plasmids and strain; *E. coli* strain DH5α and Agrobacterium tumefaciens strain EHA105 were available in our laboratory. Restriction enzymes were purchased from NEB, Cloning vector was purchased from Quanshijin, High-fidelity Taq enzyme FastPlu Fly DNA Polymerase was purchased from All Type Gold Company, T4 DNA Ligase was obtained from Thermo Corporation. Plasmid extraction kit, Yeast plasmid extraction kit, Agarose Gel DNA Recovery Kit, PCR amplification primers and DNA Marker: Hand III, DS 2000 were purchased from GENEray, BioMIGA, Suzhou Jinweizhi companies.

**Plant Material**

Using *Arabidopsis thaliana* (Col-0) as the material, total RNA was extracted according to handsome company Trizol kit, and the cDNA was obtained by inversion according to the SDR cDNA sequence. Arabidopsis seeds were surface-sterilized according to Zhou et al. (2014) protocol. The seeds were then placed on MS media, media formulation given in (Table S1), after 8-10 days on MS media, seedlings were transferred to 1/4 Hogland nutrient solution for cultivation. The young 4 ~ 5d seedlings were transferred into the soil and protected in plastic wrap Hydrates for 3 days. Sowing of Agrobacterium was based on Zhou et al. (2014). The plant flowers were transformed after full flowering stage.

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**Table S1**, 2 Res Bio • 2021 • Vol 12

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Vector Constructs

CDS sequence (1347 bp), and the SDR gene was reclamed by GENEray company reclamation kit. SDR gene was cloned with high-fidelity enzyme amplification. Primers for 35S::SDR overexpression vector were incorporated with BamHI for the F primer and Smal restriction site for R primer. While in 35S::AntiSDR (antisense) were incorporated with F/Smal and AntiSDR-R/BamHI restriction sites. The cloning vectors pEASYTM-Blunt were ligated respectively. Sequencing was done to confirm the ligated gene sequence thereafter, the recombinant plasmid was double-digested and the plant expression vector pbII121 was ligated. Double digestion was performed to verify successful ligation of the target gene. eGFP and 35S:(SDR + eGFP) vector construction was done as follows; Primers; eGFP-F and eGFP-R (supplement table S1) were used to clone the eGFP gene from pEGFP-N1. After sequencing to confirm ligation of the target insert in the cloning vector, pCAMBIA1304 was double-digested to obtain the 35S: eGFP vector. The primer SDR sub-location-F 5’/EcoRI digestion site, and SDR sub-location-R- 5’/PstI digestion site were used. After cloning the SDR gene, pEGFP-N1 was ligated, primers for (SDR + eGFP)-F were designed with (SDR + eGFP)-R/Bst EII, followed by cloning and ligation in pCAMBIA (1380 + 35S) vector to obtain 35S: (SDR + eGFP). Double digestion was performed to verify successfully ligation of the target.

Arabidopsis Transformation

Transformation followed Clough and Bent (1998) protocol. At OD 0.8, the transformed Agrobacterium was enriched, with MS liquid supplemented with 0.02% silwett-77 and 5% sucrose. Thereafter, the OD was adjusted to 0.6. Arabidopsis buds were transferred into the Agrobacterium suspension in the light-blowing constant temperature growth incubator for 30 s. After infection, the plant materials were protected with a plastic wrap to keep away light for 18-24 hours. The seeds were grown and harvested in a light constant temperature growth incubator. During screening, the seeds were grown on MS solid medium supplemented with kanamycin.

Onion Epidermal Cells Transformation

Expression vectors pCAMBIA (1380 + 35S) were constructed to obtain 35S: (SDR + eGFP) as explained previously. Onion epidermal cells with 35S::SDR+GFP were generated by agrobacterium-mediated transformation with 35S::GFP being used as a control and both were observed under spectral confocal microscope (Olympus, Tokyo, Japan) under 488 nm wavelength.

Identification of Positive Plants

DNA Level Identification

T0 seedlings DNA were obtained using primers 35S-F designed in pBI121 35S region and SDR-R inside SDR gene to identify 35S::SDR overexpression material at the DNA level by PCR; the 35S::AntiSDR antisense material was identified using 35S-F and AntiSDR-F (Table S1). The selected positive T0 seedlings were used to obtain T3 homozygotes through subsequent culture and selection.

Identification of RNA Expression Levels

T3 positive seedling transgenic material RNA was isolated; qRT-SDR-F and qRT-SDR-R were designed to identify 35S::SDR over-expression materials at the RNA level. Similarly, qRT-AntiSDR-F and qRT-AntiSDR-R were used to identify 35S:: AntiSDR antisense region. The real-time fluorescence quantitative internal reference Actin2 primers were Actin2-F and Actin2-R (Table S2-S4) illustrates the primers used. Protocols followed Quanshijin Biological cDNA synthesis kit. The reaction program was at: 42°C, 30 min.

Bioinformatics and Phylogenetic Analysis

For gene structure, protein domain and evolutionary tree analysis. SDR (At5g15710) gene structure information was retrieved from the Arabidopsis database (http://www.arabidopsis.org/index.jsp), protein sequence, and protein domain were obtained from the NCBI website (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) BLAST tool was used to predict the sequence. And, MEGA5.1 was used for tree construction. Promoter cis-element; the 1181bp of SDR upstream promoter sequence was searched for in Arabidopsis data and plant cis-acting regulatory DNA elements cis-acting element (PLACE) were used to make predictions in the http://www.dna.affrc.go.jp/PLACE/signalscan.html

Tissue Expression Pattern and Protein Sub-cellular Localization of SDR in Plants

Transformed onion epidermal were observed under a fluorescence microscope. Green fluorescent protein (GFP), GFP fluorescence; Bright, bright-field image merged GFP and bright-field image; DAPI, DAPI staining of nucleus image. Scar bar =20 μm.

SDR Response to Salt and Drought Assay

The total protein of T3 generation homozygous transgenics were extracted, and the expression level of the target protein in transgenic plants was detected by Immunoblotting following Chen et al. (2012) protocol.

Seed Germination Rate, Cotyledon Greening and Root Length Measurement

Sterilised lines of both wild-type and transformed seeds were grown with 30-40 seeds in three replicates to ensure enough production of seeds. The experiment was repeated 3 times. It was carried out in the dark and protected from vernalization for 2-3 days, at 22°C. Later, transferred to16 h / 8 h light / dark photoperiods with 100 μE m-2 s-1, light intensity. The seed germination rate was measured based on when exposed radicle and both cotyledons turned green the main root length was
measured at salt concentration of (0 ~ 200mM). Measurement was done from 2-10 days.

### Identification of Sensitivity of 35S: SDR and 35S: AntiSDR Plants to Salt Stress

Surface sterilized seeds were germinated on 1/2 MS medium (1% sucrose) containing 0 mM, 100 mM, 150 mM, and 200 mM NaCl, respectively. After treatment with salt, 35S: SDR, 35S: AntiSDR, and wild-type seeds germination rates and cotyledon greening were measured at the 4th and 7th day. Further, germination rates of wild-type and 35S: SDR plants at 200 mM NaCl and wild-type and 35S: AntiSDR plants at 150 mM NaCl were counted for 1-7 days (Figures 2-5 and S1).

### Main Root Lengths of wild-type, 35S: SDR (or OE) and 35S: AntiSDR (or AS) Under Salt Stress

Wild-type, 35S::SDR and 35S::AntiSDR seeds were germinated on MS media containing 0, 100, 150 and 200 mM NaCl for 10 d. Seedling root length of the indicated genotypes was measured at 10th d, root length was compared with that on control NaCl-free medium.

### Evaluating Sensitivity of 35S: SDR and 35S: AntiSDR Plants to Drought Stress

Sensitivity of 35S: SDR plants and 35S: AntiSDR plants to drought stress was assessed as follows; 35S: SDR plants and wild-type three-week-old Arabidopsis thaliana were not watered for 14 days, and re-watering was resumed for 3 days consecutively. On 35S: AntiSDR plants and wild-type three-week-old Arabidopsis thaliana. Plants were not watered for 14 days and watering was introduced after 14 day for 3 consecutive days.

### Statistical Analysis

All experiments were replicated three times and statistical analyses were performed using SPSS and Excel, and P<0.05 were considered to be statistically significant.

### RESULTS AND ANALYSIS

#### Promoter and Cis Element Analysis

Schematic structures of SDR protein with green bar depicting the F-box motif whereas yellow hexagon indicate Kelch domain.

The SDR protein has 448 amino acid residues (Table S3). It is predicted from the NCBI protein domain that the N-terminal (107-147) of the SDR contains an F-box motif and the C-terminal (189-233) contains a Kelch motif (Figure 1A). Classified under the C5 sub-family of the Arabidopsis F-box protein family (Gagne et al., 2002), the protein is predicted to have E3 ubiquitin ligase activity. SDR is in the same sub-family as HWS (González-Carranza et al., 2007) and LCR (Song et al., 2012). The function of SDR is unknown at present, and further research is needed. Both the protein sequence and protein domain were obtained with the aid of BLAST tool in the NCBI and protein domain prediction website (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) respectively. Transcriptional regulators can regulate gene expression through cis-acting elements and binding, so understanding the cis-acting elements in the promoter region upstream of a gene is of great significance for studying the function of genes. The 1181 bp sequence upstream of the gene was intercepted to the PLACE website to predict that there are many known functional stress response elements in the SDR promoter region (Table S2). These elements include 8 ABA-responsive elements, 22 heat shock-responsive elements, 3 dehydration-responsive elements, 3 pest and disease and salt stress response elements (pathogen and salt responsive) and 8 dehydration and cold responsive elements. The above prediction results indicate that SDR may be related to plant regulation of stress.

#### AtSDR Homology Alignment

The 694 F-box proteins have been identified in Arabidopsis (Gagne et al., 2002), and most of their functions are still being studied. In order to study the conservation of AtSDR among species, NCBI’s Blast tool was used to perform homology alignment on the amino acid sequences of SDR proteins. In Arabidopsis (At), Arabidopsis (Al), Poplar (Pt), Cucumber (Cs), Potato (St), Soybean (Gm), Alfalfa (Mt), Maize (Zm), Rice (Os) SDR homologues were found (Figure 1B), indicating that SDR is more conserved.

Figure 2 (A) below is a comparison of the derived amino acid sequences of AtSDR in nine species coloured by GENEDOC software. (At, Arabidopsis thaliana, NP_197075.1; Al, Arabidopsis lyrata, XP_002871679.1; Gm, Glycine max, XP_003552622.1; St, Solanum tuberosum, XP_00638323.1; Pt, Populus trichocarpa, XP_006384258.1; Cs, Cucumis sativus, XP_004156221.1; Zm, Zea mays, NP_001132079.1; Mt, Medicago truncatula, XP_003621585.1; Os, Oryza sativa, EEC78214.1). Two conserved domains and potential protein binding sites are marked with red line.

#### Phylogenetic Analysis

The inter-species evolution tree was constructed using the aligned homologous sequences using MEGA5 (Hall et al., 2013). AtSDR and AISDR species were found to be the closest on evolutionary relationship (Figure 3A). Phylogenetic analysis of SDR in eleven species was carried out. The dendrogram was conducted in CLUSTALX software. The tree in (Figure 3A) was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree.
Tissue Expression Pattern and Protein Sub-cellular Localization of SDR in Plants

The results in (Figure 4 A and B) showed that SDR is constitutively expressed in Arabidopsis, including seedlings, 14-day aerial shoots and roots, 40-day roots, stems, Fruit pods (silique), rosette leaves, cauline leaves and flowers. The experiment showed that at5g15710 protein is predominantly located in the nucleus in plant cells as individuals transformed with 35S:SDR+GFP exhibited green fluorescence in the nucleus of onion epidermal cells Figure 4 (C).

A: Real-time PCR of SDR under stress conditions. Two-week-old wild-type plants grown on MS agar medium were exogenously treated with 250 mM NaCl and 200 μM and for drought and heat stress, three-week-old plants grown in 1/2 Haogland culture solution were placed on filter paper for 0, 6, 12 h, treated with 37 °C for 0, 2, 4 h. B: Tissue specific expression of SDR. Seedling of 7 day-old; 14 day-old root and shoot; 40 day-old root, stem, flower, rosette leaf and cauline leaf were taken for SDR RT-PCR assay. Values are means of three replicate assays (± SD). C: Sub-cellular localization of SDR Pro35S:(SDR+ GFP) and Pro35S:GFP (control) were introduced into onion epidermal cells using.

Figure 2: Comparison of the derived amino acid sequences of AT5G15710 in nine species coloured by GENEDOC software. (At, Arabidopsis thaliana; Al, Arabidopsis lyrata; Gm, Glycine max; Mt, Medicago truncatula; Pt, Populus trichocarpa; Cs, Cucumis sativus; St, Solanum tuberosum; Zm, Zea mays; Os, Oryza sativa). Two conserved domains and potential protein binding sites are marked with red line. (Figure 2)
Agrobacterium tumefaciens infection, and then observed under a fluorescence microscope. Green fluorescent protein (GFP), GFP fluorescence; Bright, bright-field image; Merge, merged GFP and bright-field image; DAPI, DAPI staining of nucleus image. Scar bar = 20 μm. Vertical bars represent the standard deviation of the mean (n = 100), and significant differences are indicated by asterisks (P < 0.05).

Identification of Sensitivity of 35S: SDR and 35S: AntiSDR Plants to Salt Stress

Because SDR can be induced by salt, it is likely that SDR is involved in the regulation of plant salt stress, and transgenic plants may change the plant’s response to salt stress. According to previous work by Xiong et al. (2002), we based on his methodology to observe the growth response of 35S: SDR, 35S: AntiSDR and wild type under salt stress. Surface sterilized seeds were sown in 1/2 MS medium (1% sucrose) containing 0 mM, 100 mM, 150 mM, and 200 mM NaCl, respectively. After treatment with salt, 35S: SDR, 35S: AntiSDR, and wild-type seeds were counted; during germination, growth after germination. The results are shown (Figure 5). On 150 mM NaCl treatment, the germination rate of 35S: SDR plants (77.77%, 87.62%) was higher than that of wild type (Figure 5B), while the germination rate of 35S: AntiSDR plants (4.16%, 4.2%) was lower than that of wild type (Figure 5E). Similarly, 35S: SDR plants turned green (21.7%, 25.71%) higher than the wild type map (Figure 5C), 35S: AntiSDR plants turned green...
Figure 5: Seeds of Col-0, 35S::SDR, and 35S::AntiSDR genotypes were grown on medium containing different concentrations of NaCl. Photographs (A, C) were taken for 7-d-old seedlings Picture of Cotyledon green and Germination rates (B, D) were measured after 4 d and cotyledon greening (E, F) were measured after 7 d. Vertical bars represent the standard deviation of the mean (n = 100 seedlings), and significant differences are indicated by asterisks (P < 0.05). (Pictures aligned and petri plates labelled)
(2.78%, 1.85%) lower than Wild type (Figures 5F). In addition, the germination rates of wild-type and 35S: SDR plants at 200 mM NaCl and wild-type and 35S: AntiSDR plants at 150 mM NaCl were counted for 1-7 days (Figures 5G, H).

**Growth Responses of Wild-type, 35S:SDR (OE lines) and 35S:AntiSDR (AS lines) Plants to Salt Stress**

Seeds of wild-type, 35S:SDR and 35S:AntiSDR plants were germinated on the plates containing the different concentrations of NaCl. (A,D) Picture of Cotyledon green and Germination rates (B,E) were measured after 4 d and cotyledon greening (C,F) were measured after 7 d and Germination after sowing on the plate from 1~7d were measured (G,H). Vertical bars represent the standard deviation of the mean (n = 100 seedlings). Changes in main root length under salt stress were also measured. Without treatment, the root length of 35S: SDR plants was shorter than that of wild type, and the root length of 35S: AntiSDR plants was longer than that of wild type. Interestingly, after salt treatment, it was found that the root length of 35S: SDR plants was longer than that of wild type (Figure 6A), in contrast, the root length of 35S: AntiSDR plants was shorter than that of wild type (Figure 6B). Results showed that 35S: SDR plant root length was inhibited less than wild type, and 35S: AntiSDR plant root growth was inhibited more than wild type. These results indicate that 35S: SDR plants are resistant to salt, while 35S: AntiSDR plants are sensitive to salt.

**Identification of Sensitivity of 35S: SDR and 35S: AntiSDR Plants to Drought Stress**

Experimental data show that SDR expression is down-regulated under drought stress, and that SDR is involved in plant responses to salt stress. In plants, salt stress also produces osmotic stress, which is physiologically related to drought stress (Zhang _et al._, 2007). Therefore, the sensitivity of 35S: SDR plants and 35S: AntiSDR plants to drought stress was further identified, 35S: SDR plants and wild-type three-week-old _Arabidopsis thaliana_ were not watered for 14 days, and re-watering was resumed for 3 days both 35S: SDR plants and wild-type lost water and withered. It was found that 80% of wild-type plants were resurrected, while only 20% and 10% 35S: SDR plants were resurrected (Figure 7A, B). On 35S: AntiSDR plants and wild-type three-week-old _Arabidopsis thaliana_. Plants were not watered for 14 days and watering was introduced after 14 day for 3 consecutive days. The result showed most of the 35S: AntiSDR plants and wild type withered. Reviving only 10% of the wild-type plants in comparison to 70% and 60% of the 35S:
AntiSDR plants revival (Figure 7C, D) concluding that 35S: SDR plants are more sensitive to drought stress, while 35S: AntiSDR plants are drought tolerant.

DISCUSSION

F-box proteins roles have been significant in regulation of various developmental processes and stress responses involving most plant hormone signalling pathways (Hwang et al., 2020). For instance, in rice, only three F-box protein functions have been described. GID2 (GA-insensitive dwarf 2), the first F-box protein identified in rice involved in gibberelllic acid signaling positive regulation with D3 (dwarf 3) F-box proteins being involved in tiller bud activity and MAIF1 (miRNAs regulated and abiotic stress induced F-box gene) has been hypothesized to play a negative role in the response to abiotic stresses by regulating root growth. However, despite their known vital roles in plant development and responses to abiotic stress majority of F-box proteins roles in rice remain unknown. In Arabiodpsis, under drought stress, the F-box protein DOR inhibits ABA-induced stomatal closure (Bu et al., 2014). Another F-box protein AtFBP7 is highly essential under temperature stress according to Calderón-Villalobos et al. (2007). In various studies, it has been illustrated that several F-box protein gene products are involved in plants survival under abiotic stresses. In rice, 23 F-box proteins are expressed upon exposure to saline stress. The F-box protein, CarF-box1, has been found to be up regulated by salt and drought constraints in chickpea (Jain et al., 2007; Guerra et al., 2012; Zhang et al., 2019; Hwang 2020). The response of Phaseolus vulgaris against wound stresses and osmotic changes and the application of methyl jasmonate (MeJA), salicylic acid (SA) and ABA is due to the accumulation of mRNA from PvFBS1, a putative F-box gene (Maldonado-Calderó et al., 2012). Overexpression of the MAIF1 gene in rice reduces abiotic stress tolerance and promotes root growth (Yan et al., 2011). TdRF1, a wheat RING ubiquitin ligase, is the building block against cellular dehydration as highlighted by Zhang et al. (2017).

In our experiment, after treatment of plants with salt at concentrations; improved germination, increased root growth, and high chlorophyll content of transgenic plants under salinity suggested that SDR overexpressing showed improvement in salt stress tolerance. The ability to maintain photosynthetic stabilization is essential to salt acclimation and involves phenotypic plasticity mechanisms (Hauvermale and Marwa...
Plants have developed a complex molecular and cellular regulatory mechanisms for regulating salt and drought stress. Both salt and drought stress leads to water shortage and oxidative stress in plants illustrating an overlap in regulation mechanism of salt and drought stress. However, the adaptive regulation of plants to salt and drought can be divided into three aspects according to Chen et al. (2012) and Marques et al. (2017). First, the reconstruction of the homeostasis mainly includes ionic homeostasis and osmotic homeostasis caused by salt stress. Plants regulate ion homeostasis mainly through a series of related ion channels in cells. When plant cells experience high external salt stress, the intracellular calcium ion concentration increases, and calcium signal activates calmodulin (SOS3). SOS3 can bind to a serine/threonine protein kinase, SOS2, and the kinase complex is activated and phosphorylates SOS1. SOS1 encodes a Na⁺/H⁺ antiporter that is located on the plasma membrane. When Na⁺ concentration increases, and calcium signal activates calmodulin through a series of related ion channels in cells. When plant cells sense a Na⁺ gradient, SOS1 is activated to transport Na⁺ ions outside the cell.

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### SUPPLEMENTARY

#### Table S1: Primers used for vector construction

| Primer name      | Primer Sequence (5’~3’)                           |
|------------------|---------------------------------------------------|
| SDR-F            | CGGGATCCATGGAGCTTAGGATTTTG                         |
| SDR-R            | TCCCCGCGGTCAAGGACAGATGCATC                         |
| AntiSDR-F        | TCCCCGCGGTCAAGGACAGATGCATC                         |
| AntiSDR-R        | CGGGATCCATGGAGCTTAGGATTTTG                         |
| SDR Sublocation-F | TCCCCGCGGTCAAGGACAGATGCATC                        |
| SDR Sublocation-R | CGGGATCCATGGAGCTTAGGATTTTG                       |

#### Table S2: Primers used for gene expression

| Primer name      | Primer Sequence (5’~3’)                           |
|------------------|---------------------------------------------------|
| qRT-SDR-F        | AACTCTTTTCGCTCATCTTGGT                            |
| qRT-SDR-R        | ACTTGGTGCAAATCTTACT                              |
| qRT-AntiSDR-F    | TCTCGGTGGTACATCTTACT                             |
| qRT-AntiSDR-R    | TCTCGGTGGTACATCTTACT                             |
| qRT-HKT1-F       | TCAGTGCATATGGAAACGTGGG                           |
| qRT-HKT1-R       | CAGCCACATCGTGGAT                              |
| qRT-PSCS1-F      | AGCGAGCTGAATGCAAGATGG                          |
| qRT-PSCS1-R      | AAGTGCAGCTGTGGTGTGTCG                          |
| qRT-RD29A-F      | GTTACTGATCCACCAAAAGAGA                          |
| qRT-RD29A-R      | GGAGAGTGCTGACTGCTACTCCA                       |
| qRT-COR15B-F     | TCAGTGGCATGATGGCTCTT                            |
| qRT-COR15B-R     | TCTCGGTCCGCTAGTTCGACATGTCG                      |
| qRT-KIN1-F       | TGGAGCTGGAGCAGAACAA                           |
| qRT-KIN1-R       | GACCCGAAATCGTGCCTGGTTC                        |
| Actin2-F         | CATCAGAAGAGACATCTGGAC                          |
| Actin2-R         | GATGGAACTGACTGCTCAC                          |
Table S3: Genomic sequence and protein features of SDR (At5g15710)

(A) Full length genomic DNA and CDS

| ATCTATCTTCTTCTCTTGTTACTAAAAAGGACGAAGCTTGTTGCATAATATGTTGAGGTAAATTACTAATTACTGATCCAAAGTTCGAATCTTTGCTCCAACT | GATACTTCTGTTTGGTCGATGATCGAGTGATCTTCGTTGGGTTTTGGGGATCTAAGTCGTCTATATAGCTAATGGTTTGGATTTGAGTTTGAATGACGGAAGCT | GAGCGTTTAATG | GAGCGTTTAATG | GAGCGTTTAATG | GAGCGTTTAATG | GAGCGTTTAATG | GAGCGTTTAATG |
| ATCTCTCTCTTCTTCGTGTTACTAAAAAGGACGAAGCTTGTTGCATAATATGTTGAGGTAAATTACTAATTACTGATCCAAAGTTCGAATCTTTGCTCCAACT | GATACTTCTGTTTGGTCGATGATCGAGTGATCTTCGTTGGGTTTTGGGGATCTAAGTCGTCTATATAGCTAATGGTTTGGATTTGAGTTTGAATGACGGAAGCT | GAGCGTTTAATG | GAGCGTTTAATG | GAGCGTTTAATG | GAGCGTTTAATG | GAGCGTTTAATG | GAGCGTTTAATG |

The green letters represent CDS (1347 bp). Sequences highlighted in blue indicate translation start (ATG) and stop (TAG) sites.

(B) Protein sequence of SDR (AT5g15710)

MERLGFWGLLMGSVEKSLDSGNSLACSASAKNGDEESSTSSKQVSPLKGSGSRNTSPLGRVG | SRNTSPSRQKV | KTKPRGLEEETVASFGKQVADV | QMEDGIWAM | PEDOLLNEILARVPPFMIFRISVCKWKVNLLQNSFLKFR | NVSVSHGPCLLTFWKNSQPQCSVSPSLPKEWYWIFFPFILPWPWAFVLV | GSSGGLVCSFLGDLTFRTLVCNPLLMSWRTLPSPHYNQQRQVIMVORSKFPSVATSDIYGDSELSPTEVYDSKTDWLSLHIMPANLCSCKMAYC | DSRLLYLETSLPGLMYRLSDGQWHIPKPFRSLDLLGYLVAQTKRLFLVGRGLYQLSTQLSQRMLWEHDHTKSVWVEISRPKYPFRALLRSLAERFEC | FGDNLIFSTWSQNGKGLLYNVDKWSWSSGCAUSCNQSCNVCFYEPFRASDVL |

The green letters represent CDS (1347 bp). Sequences highlighted in blue indicate translation start (ATG) and stop (TAG) sites.

Table S4: Cis-Regulatory Elements of promoter of SDR

| Name                  | Consensus     | Functions                     | Number |
|-----------------------|---------------|-------------------------------|--------|
| EBOXBNNAPA            | CANNTG        | Abscisic acid responsive       | 8      |
| CBFHV                 | RYCGAC        | Dehydration responsive         | 3      |
| ARFAT                 | TGCTCT        | Auxin responsive               | 1      |
| ARRI1AT               | NGATT         | Response regulator             | 15     |
| CURECORECR            | GTAC          | Copper responsive              | 2      |
| CCAATBOX1             | CCAAT         | Heat responsive                | 22     |
| PYRIMIDINEBOXOSRAMYIA | CTTTTT        | GA responsive                  | 3      |
| GT1CONSUS             | GRWAAN        | Light responsive               | 8      |
| GT1MASCAM4            | GAAAAA        | Pathogen and salt responsive   | 3      |
| IBOXCORE              | GAAAAA        | Pathogen and salt responsive   | 3      |
| INRNTPASDB            | YTCANTY       | Light-responsive               | 4      |
| MYB1AT                | WAAACCA       | Abscisic acid responsive       | 3      |
| MYCONSENSUSAT         | CANTG         | Dehydration and cold responsive| 8      |

Sequence selected for SDR antibodies is highlighted with purple and F-box motif is highlighted with yellow.
Figure S1: Germination rate of seeds of transgenic lines and wild type. 
A: Germination rate of seeds of 35S::SDR and wild type sown on MS containing 200 mM NaCl. B: Germination rate of seeds of 35S::AntiSDR and wild type sown on MS containing 150 mM NaCl. The germination rate was measured from 1 day to 7 day. Vertical bars indicated SD of three replicates (n>100).