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Research Article

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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

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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 constrains 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 additionally, 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, karrikin signaling, temperature signaling, and senescence pathways (Brewer *et al*., 2013). 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 karrikin 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 FastPfu Fly DNA Ploymerase 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) protocal. 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 *Agrobaterium* was based on Zhou *et al.* (2014). The plant flowers were transformed after full flowering stage.

Vector Constructs

CDS sequence (1347 bp), and the SDR gene was reclaimed 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 SmaI restriction site for R primer. While in 35S::AntiSDR (antisense) were incorporated with F/SmaI 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 pBI121 was ligated. Double digestion was performed to verify successful ligation of the target gene. eGFP and 35: :(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 35: 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 35: (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 35: :(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 werw 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

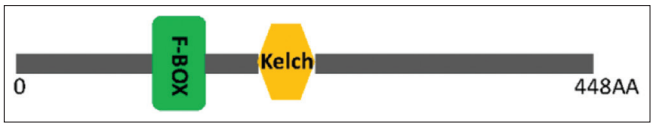

Figure 1: AtSDR protein structure

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_006338323.1; 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 AlSDR species were found to be the closest on evolutionary relationship (Figure 3 A). 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.

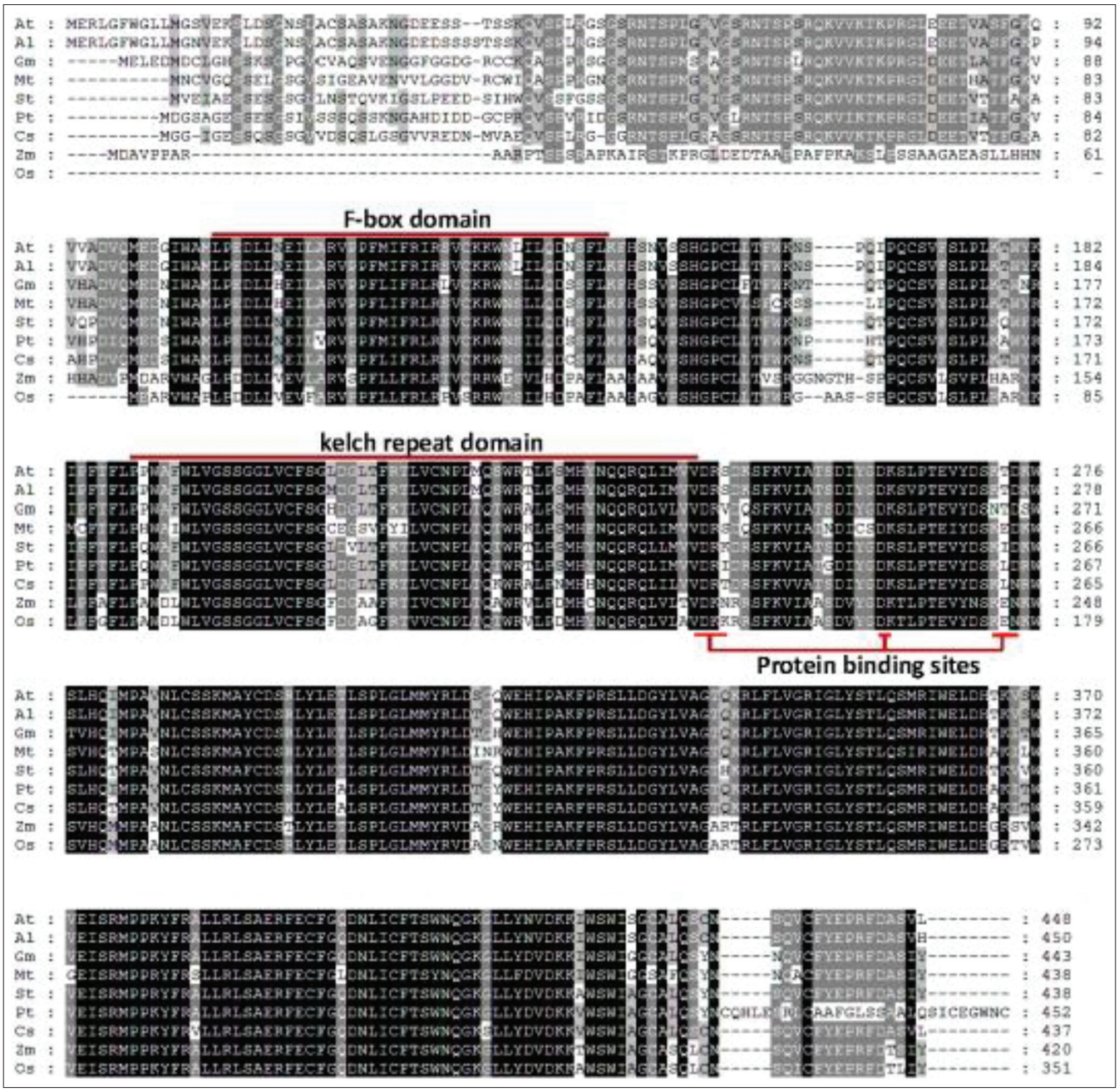

Figure 2: Comparison of the derived amino acid sequences of *AT5G15710* in nine species coloured by GENEDOC software. (At, Arabidopsis thaliana; Al, Arabidopsis lyrata; Gm, Glycine max; Mt, *Medicago truncatula*; Pt, *Populus trichocarpa*; Cs, *Cucumis sativus*; St, *Solanum tuberosum*; Zm, *Zea mays*; Os, *Oryza sativa*).Two conserved domains and potential protein binding sites are marked with red line. (Figure 2)

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-celluar localization of *SDR* Pro35S:(*SDR*+ GFP) and Pro35S:GFP (control) were introduced into onion epidermal cells using

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 ($P < 0.05$).

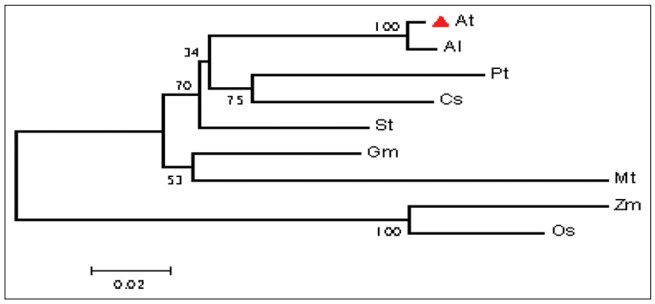

Figure 3: Phylogenetic analysis of *AT5G15710* in eleven species. The dendrogram was conducted in CLUSTALX software

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

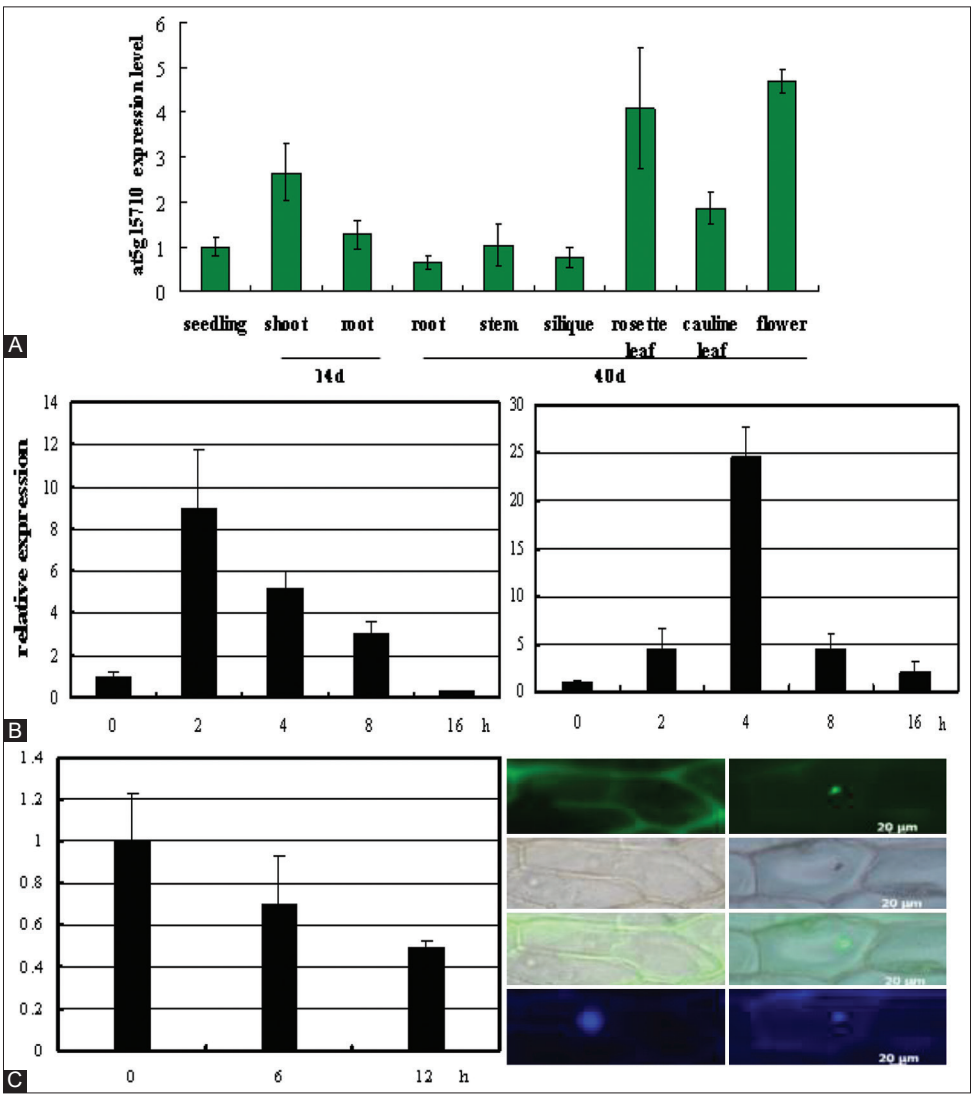

Figure 4: Transcriptional expression of *SDR* under the abiotic stress (A), Tissue specific expression of *SDR* (B), Sub celluar localization of *SDR*(C)

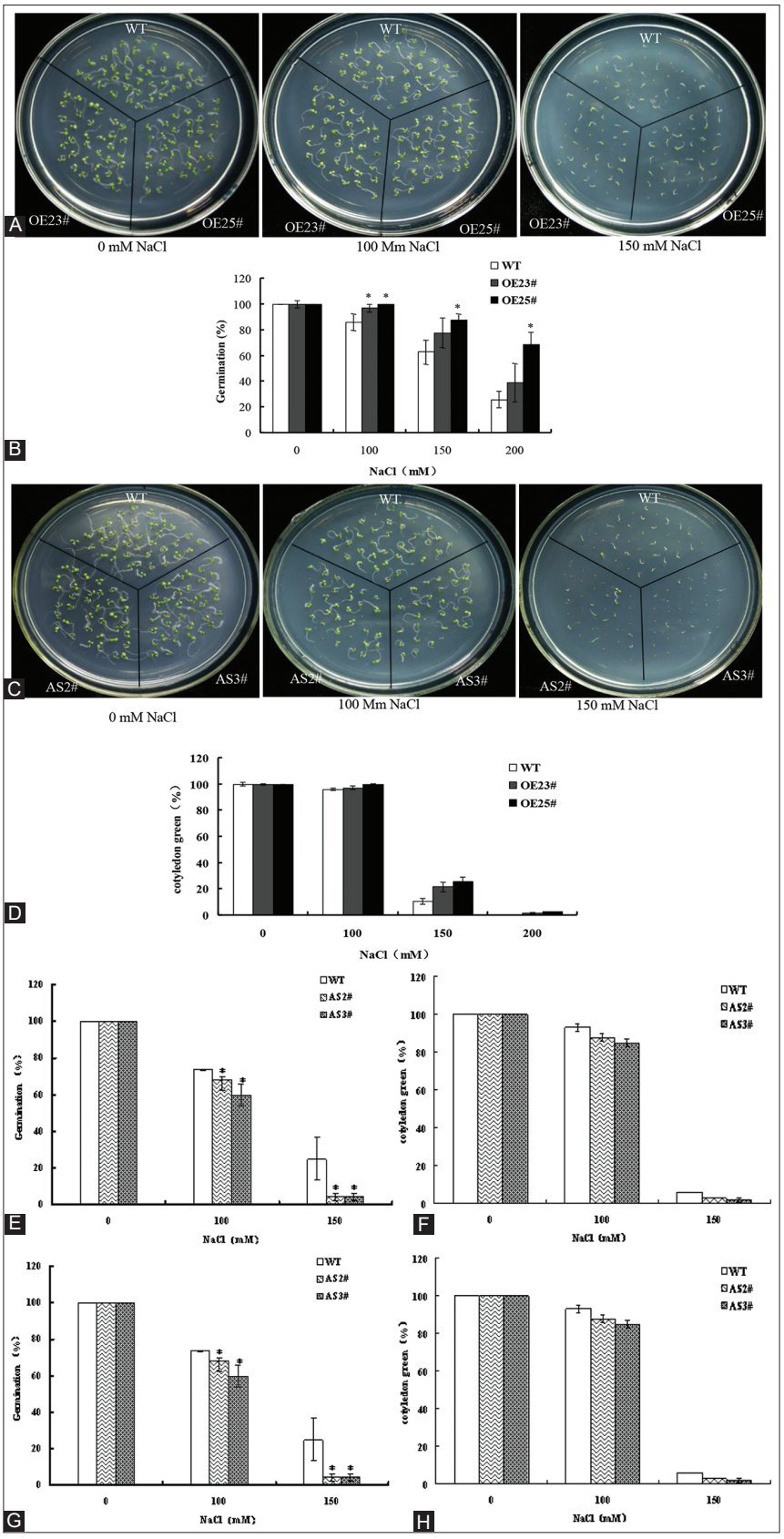

Figure 5: Seeds of Col-0, *35S::SDR*, and *35S::AntiSDR* genotypes were grown on medium containing different concentrations of NaCl. Photographs (A, C) were taken for 7-d-old seedlings Picture of Cotyledon green and Germination rates (B,D) were measured after 4 d and cotyledon greening (E,F) were measured 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:

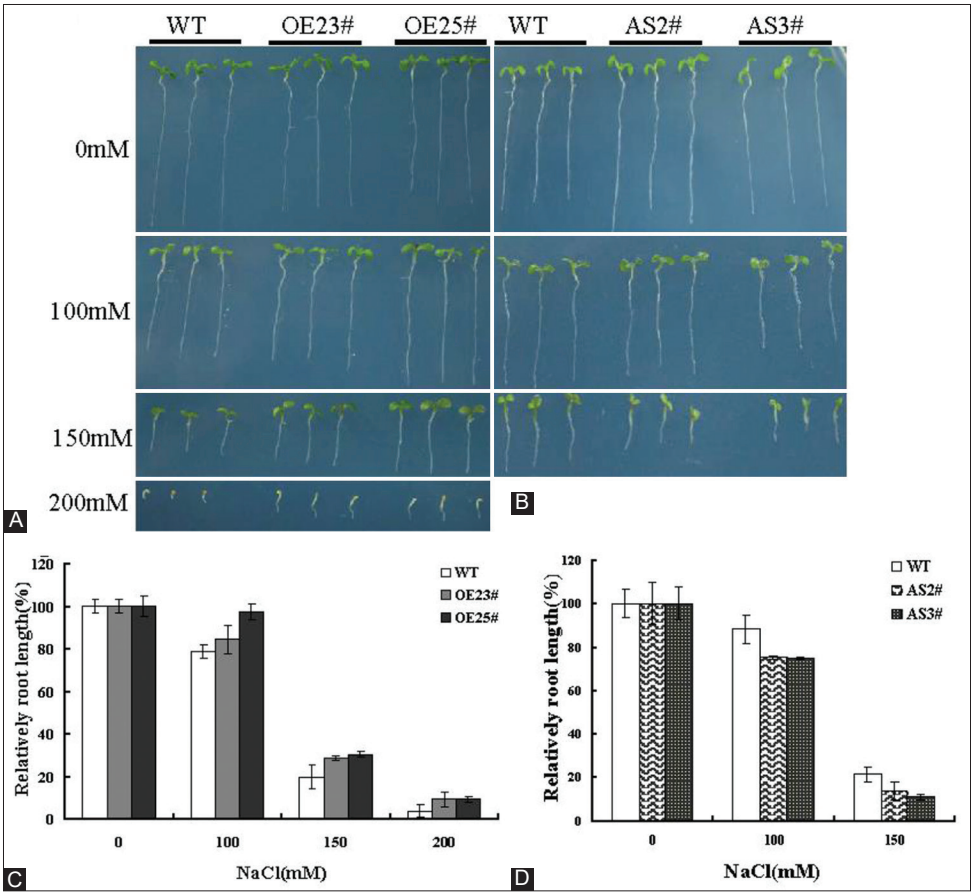

Figure 6: (A,B) Root growth of wild-type, *35S::SDR* and *35S:AntiSDR* plants on MS medium containing 0, 100, 150 and 200 mM NaCl. Seeds of the plants were germinated for 10 d on the MS medium with or without NaCl, and representative plants are shown. (C,D) Root growth measurements. Seedling root length was measured at 10 d, root growth compared with that on NaCl-free medium is indicated. Vertical bars represent the standard deviation of the mean (n =100 seedlings), and significant differences are indicated by asterisks ($P < 0.05$)

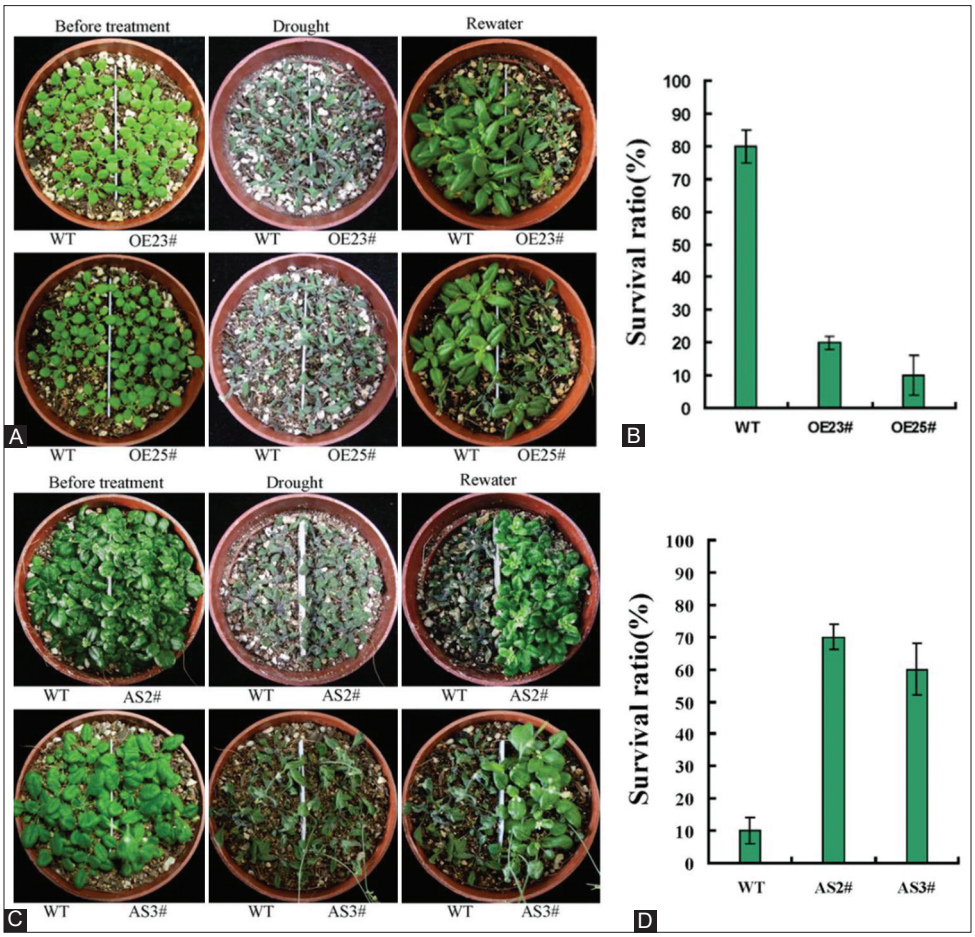

Figure 7: A/C: Three week-old wild-type, *35S::SDR* (A) and *35S::AntiSDR* (C) plants were dehydrated for 12(A) or 14 (C) d, followed by re-watering for 3 d. Dehydration tolerance was assayed as the ability of plants to resume growth when returned to normal conditions. B/D: Survival of wild-type and *35S::SDR* (B) or *35S::AntiSDR* (D) plants by re-watering for 3 d after dehydration treatment for 14 d (B) or 12 d (D). Vertical bars represent the standard deviation of the mean (n =100 seedlings), and significant differences are indicated by asterisks ($P < 0.05$)

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 gibberellic 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 Arabidopsis, under drought stress, the F-box protein DOR inhibits ABA-induced stomatol 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 changyanes 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

2019). AntiSDR plants exposed to increasing levels of NaCl showed a diminished net photosynthetic rate (Pn), accompanied by a significant decrease in stomatal conductance (Gs) and transpiration rate (E). But the transgenic plants showed higher photosynthetic rates under salt stress than the WT plants, which may be due to the high levels of photosynthetic pigments and RWC suggesting there was less damage to the photosynthesis machinery in the transgenic plants. Our findings were in line with Bu *et al*. (2014) work.

Again, this work was in line with our other experiment (unpublished) where the expression of genes related to salt stress and drought stress in 35S: SDR plants and wild-type plants for three weeks of hydroponic cultivation was quantitatively detected by real-time fluorescence. The results showed that in 35S: SDR plants, the expression of *HKT1* related to salt stress was significantly decreased, while the expression of *P5CS1* was increased while expression of drought stress related genes *RD29A*, *COR15B*, and *KIN1* was down-regulated. These results indicate that SDR is a positive regulator for salt stress and a negative regulator for drought stress in plants. However, the specific molecular mechanism of SDR in regulating abiotic stress is unclear.

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 cell membrane and is responsible for transporting sodium ions inside the cell to the outside. At the same time, the activity of HKT1, an ion channel that absorbs sodium ions, is inhibited Chen *et al*. (2012) to rebuild the low sodium ion environment in the cell.

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SUPPLEMENTARY

Table S1: Primers used for vector construction

Primer name	Primer Sequence(5′~3′)
SDR-F	CGGGATCCATGGAGCGTTTAGGATTTTG
SDR-R	TCCCCCGGGTCAGAGGACAGATGCATC
AntiSDR-F	TCCCCCGGGATGGAGCGTTTAGGATTTTG
AntiSDR-R	CGGGATCCTCAGAGGACAGATGCATC
SDR Sublocation-F	TCCCCCGGGATGGAGCGTTTAGGATTTTG
SDR Sublocation-R	CGGGATCCGAGGACAGATGCATCAAATC

Table S2: Primers used for gene expression

Primer name	Primer Sequence(5′~3′)
qRT-SDR-F	AACTCTTTCGCCTCTTGGT
qRT-SDR-R	ACTTTGGTGGCATTCTACT
qRT-AntiSDR-F	TTTCCGGTTGTGCTCTT
qRT-AntiSDR-R	TTGCTGGCGAACTTGTA
qRT-HKT1-F	TCAGTGCATATGGAAACGTTGG
qRT-HKT1-R	CAGCCACCATCGCTGATG
qRT-P5CS1-F	AGCAGCCTGTAATGCGATGG
qRT-P5CS1-R	AAGTGACGCCTTTGGTTTGC
qRT-RD29A-F	GTTACTGATCCCACCAAAGAAGA
qRT-RD29A-R	GGAGACTCATCAGTCACTTCCA
qRT-COR15B-F	TCAGTGGCATGGGTTCTT
qRT-COR15B-R	TCCTCAGTCGCAGTTTCA
qRT-KIN1-F	TGGAGCTGGAGCACAACA
qRT-KIN1-R	GACCCGAATCGCTACTTGTTC
Actin2-F	CATCAGGAAGGACTTGTACGG
Actin2-R	GATGGACCTGACTCGTCATAC

Table S3: Genomic sequence and protein features of SDR (At5g15710)

(A) Full length genomic DNA and CDS

ATCTCTCTCTTCTTCGTGTTACTAAAAAGGACGAAGCTTGTTGCATAATATGTTGAGGTAAATTACTAATTACTGATCCAAAGTTCGAATCTTTGCTCCAACT
CCAGGCTAGCTGATTGCGTAGCTTCCGATTGATTTCTACCTGAGTTTTGAGTTCCTTTGTGGCCACTTCGTTGTTCTTCTGCTGGGTTTTTTGCTCGAGGATCT
GATACTTCTGTTTGGTCGATGATCGAGTGATCTTCGTTGGGTTTTGGGGATCTAAGTCGTCTATATAGCTAATGGTTTGGATTTGAGTTTGAATGGAGCGTTTA
GGATTTTGGGGATTGCTAATGGGTAGTGTGGAAAAGTCATTGGATTCTGGAAATTCGTTGGCTTGCTCTGCATCTGCTAAGAATGGAGACGAAGAGAGTAGT
ACTTCATCGAAGCAAGTTTCACCATTGAAGGGTTCTGGGTCGAGAAATACTAGTCCTTTAGGTCGAGTTGGGTCGAGAAACACGAGTCCTTCTAGGCAGAA
AGTGGTGAAGACGAAGCCTCGTGGTCTAGAGGAAGAAACAGTTGCTTCATTTGGTAAACAAGTTGTTGCTGATGTGCAGATGGAAGATGGTATATGGGCAA
TGCTTCCAGAGGATTTGCTCAATGAGATTTTAGCTAGGGTTCCACCGTTTATGATATTTCGAATCCGGTCTGTTTGTAAAAAATGGAACTTGATTCTTCAGGA
TAATAGTTTTCTCAAGTTTCACTCAAATGTGTCATCTCATGGGCCTTGTCTTCTCACTTTCTGGAAGAACTCGCCGCAGATTCCGCAATGCTCAGTTTTTAGTT
TGCCATTGAAGACATGGTACAAAATTCCATTCACGTTTTTGCCTCCATGGGCTTTTTGGTTGGTTGGTTCTTCAGGTGGTCTCGTTTGTTTTTCGGGTCTTGAT
GGTCTAACTTTCAGAACTTTAGTATGCAATCCTCTGATGCAGAGTTGGAGGACTCTACCGAGTATGCACTATAACCAACAAAGGCAATTGATTATGGTCGTG
GATCGCTCAGACAAATCGTTCAAAGTCATAGCCACAAGTGATATATACGGGGATAAGTCACTTCCTACTGAAGTTTATGATTCCAAAACTGACAAATGGTCC
TTACATCAGATAATGCCTGCGGTGAACTTATGCTCCTCGAAAATGGCTTATTGTGATTCCCGGTTATATCTAGAAACTCTTTCGCCTCTTGGTTTGATGATGTA
TCGGCTTGATTCAGGGCAATGGGAACACATTCCAGCTAAATTCCCGAGATCTTTGTTGGATGGTTACTTAGTTGCTGGAACTCAGAAGAGATTGTTTCTCGT
GGGAAGGATTGGCCTCTACAGTACTCTCCAAAGCATGAGAATATGGGAGCTTGATCACACAAAGGTCTCTTGGGTAGAGATAAGTAGAATGCCACCAAAG
TACTTCCGAGCACTTCTGAGACTTTCGGCTGAGAGGTTCGAGTGTTTTGGACAAGATAATTTGATCTGCTTTACGTCTTGGAATCAAGGAAAAGGTCTTCTA
TACAATGTGGATAAGAAAATTTGGTCTTGGATTTCCGGTTGTGCTCTTCAGTCATGCAACAGCCAAGTGTGCTTTTATGAGCCAAGATTTGATGCATCTGTCC
TCTGAACAATAAGTTATCGTCTGTCTCACATCATTCTTGAAAACTTACAAGTTCGCCAGCAAAACATGTCAGAAATATGAAATCAAAGAGGGTTTGATGTGT
ACCTTCAGTGTTAATGAAGACCTGGTCAGCAATGATATGCTTCACCAATGGTTAACAATATCGAGGAGAAAAACTGTAAGATAAACTTGTTTCTAGCTTTCT
GTAAATTAGCATTCACTCGATATGAAAACTTTCTCAATA

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)

MERLGFWGLLMGSVEKSLDSGNSLACSASAKNGDEESSTSSKQVSPLKGSGSRNTSPLGRVGSRNTSPSRQKVVKTKPRGLEEETVASFGKQVVADV
QMEDGIWAMLPEDLLNEILARVPPFMIFRIRSVCKKWNLILQDNSFLKFHSNVSSHGPCLLTFWKNSPQIPQCSVFSLPLKTWYKIPFTFLPPWAFWLV
GSSGGLVCFSGLDGLTFRTLVCNPLMQSWRTLPSMHYNQQRQLIMVVDRSDKSFKVIATSDIYGDKSLPTEVYDSKTDKWSLHQIMPAVNLCSSKMAYC
DSRLYLETLSPLGLMMYRLDSGQWEHIPAKFPRSLLDGYLVAGTQKRLFLVGRIGLYSTLQSMRIWELDHTKVSWVEISRMPPKYFRALLRLSAERFEC
FGQDNLICFTSWNQGKGLLYNVDKKIWSWISGCALQSCNSQVCFYEPRFDASVL

Sequence selected for SDR antibodies is highlighted with purple and F-box motif is highlighted with yellow. SDR has molecular masses of 50.7 KD.

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	TGTCTC	Auxin responsive	1
ARR1AT	NGATT	Response regulator	15
CURECORECR	GTAC	Copper responsive	2
CCAATBOX1	CCAAT	Heat responsive	22
PYRIMIDINEBOXOSRAMY1A	CCTTTT	GA responsive	3
GT1CONSENSUS	GRWAAW	Light responsive	8
GT1GMSCAM4	GAAAAA	Pathogen and salt responsive	3
IBOXCORE	GATAA	Light-responsive	2
INRNTPSADB	YTCANTYY	Light-responsive	4
MYB1AT	WAACCA	Abscisic acid responsive	3
MYCCONSENSUSAT	CANNTG	Dehydration and cold responsive	8

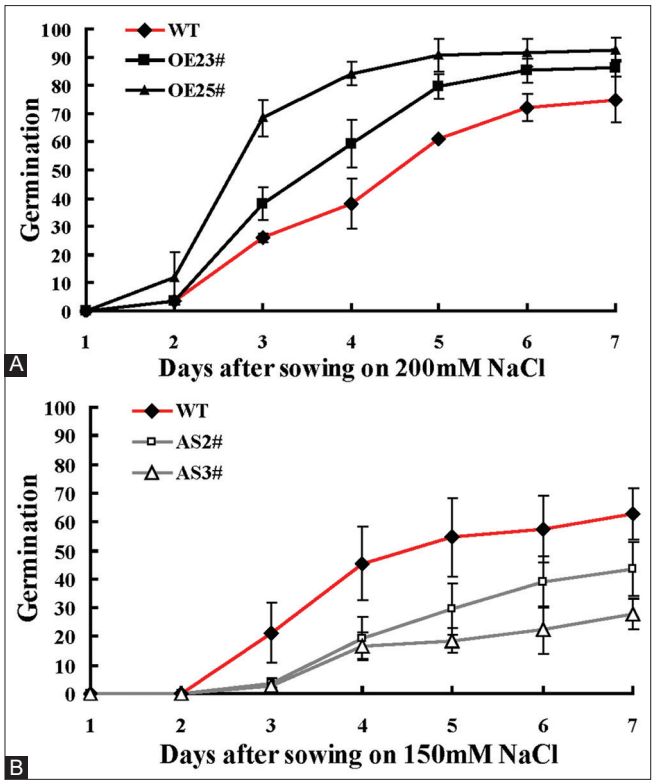

Figure S1: Germination rate of seeds of transgenic lines and wild type. A: Germination rate of seeds of *35S::SDR* and wild type sown on MS containing 200 mM NaCl. B: Germination rate of seeds of *35S::AntiSDR* and wild type sown on MS containing 150 mM NaCl. The germination rate was measured from 1 day to 7 day. Vertical bars indicated SD of three complicates (n>100).