Arabidopsis SDIR1 Enhances Drought Tolerance in Crop Plants

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Arabidopsis E3 ligase salt- and drought-induced RING-finger 1 (SDIR1) has been found to be involved in abscisic acid (ABA)-related stress signaling. SDIR1-overexpressing Arabidopsis plants exhibit improved tolerance to drought. Tobacco (Nicotiana tabacum) and rice (Oryza sativa) are two important agronomic crop plants. To determine whether SDIR1 enhances drought resistance in crop plants, SDIR1 transgenic tobacco and rice plants were generated. Ectopic expression of SDIR1 in both plants conferred improved drought tolerance ability. These results suggest that SDIR1 can function as a drought-tolerance engineering candidate gene in crop plants.

Key words: drought tolerance; rice; salt- and drought-induced RING-finger 1 (SDIR1); tobacco; transgenic

Abbreviations: CaMV, cauliflower mosaic virus; ABA, abscisic acid; ABF, ABRE binding factor; ABI5, ABA insensitive 5; HPT, hygromycin phosphotransferase; PCR, polymerase chain reaction; SDIR1, salt- and drought-induced RING-finger 1; CDS, coding sequences; Ubi, ubiquitin 1

Abiotic stress is the major cause of crop loss worldwide.1) Drought is one of the greatest abiotic stresses to agriculture, inhibiting plant growth and reducing productivity. Transgenic approaches provide considerable opportunities to improve plant drought-tolerance.2,3) Besides the strategy of introducing functional genes into plants to protect injured cells, recent studies of signaling and regulatory pathways against water deficit provide new opportunities to engineer drought-resistant plants.4–7) Previous studies have revealed that drought stress-related pathways usually fall on both the phytohormone ABA-dependent and ABA-independent signaling pathways, including cascades of signal sensing, protein phosphorylation, protein degradation, transcription regulation, etc.5,7,8) Many regulatory factors involved in drought tolerance signaling have been characterized and applied to genetic engineering, including transcription factors (ABF3 and DREB1/CFB), protein kinases (CDPK and SnRK2), etc.9–12)

Our previous results indicated that Arabidopsis E3 ligase SDIR1 is a positive regulator in the ABA signal transduction pathway and that SDIR1 overexpressing Arabidopsis plants exhibit improved tolerance to drought.13) This yields strategies to improve drought tolerance in crop plants. In this study, we engineered SDIR1 transgenic tobacco Nicotiana tabacum and rice Oryza sativa plants to determine their drought-tolerance ability.

To generate SDIR1 transgenic tobacco and rice, Arabidopsis SDIR1 was ectopically expressed in tobacco and rice plants by transformation. SDIR1 CDS was under the control of the CaMV 35S promoter for tobacco, and under the maize (Zea mays) ubiquitin 1 promoter for rice (Fig. 1A). Dozens of independent transgenic lines were screened with hygromycin and transferred into soil for T1 seeds. Most of the transgenic lines showed no visible changes in phenotype under standard growth conditions, in contrast with wild-type plants. By segregation analysis of the hygromycin gene in T2 progenies, transgenic lines with no further segregation were selected for deeper analysis. To determine the expression levels of the SDIR1 transgene in transgenic tobacco and rice, RNA gel blot analysis was performed using an Arabidopsis SDIR1-specific probe.13) Under unstressed conditions, SDIR1 transcripts were detectable at different expression...
levels in the 80% transgenic lines, but not in the pCAMBIA-transformed control plants (a partial blot is shown in Fig. 1B). Homozygous T2 lines with SDIR1 high expression levels, as indicated in Fig. 1B, were selected for further analysis.

The SDIR1 gene has been found to be a drought-induced gene in Arabidopsis, and SDIR1 overexpressing Arabidopsis plants show enhanced drought-tolerance capacity. To determine whether Arabidopsis SDIR1 plays a role in drought-tolerance in tobacco, 35S-SDIR1 transgenic tobacco plants were subjected to drought stress treatment together with vector transgenic controls. After progressive dehydration for 15 d, control plant leaves were rolled and wilted, while SDIR1 transgenic tobacco leaves appeared healthy, and only older leaves exhibited a little rolling (Fig. 2A). When dehydration was prolonged to 28 d, all the plants showed completely inhibited growth. Then they were rewatered for recovery analysis. After 2 d, 30% of the 35S-SDIR1 plants recovered, while none of control plants did, and after 10 d of rewatering, near 60% 35S-SDIR1 plants survived well and grew, and in contrast less than 30% of the control plants recovered from dehydration (Fig. 2B). These results indicate that Arabidopsis SDIR1 can increase drought tolerance in transgenic tobacco.

To determine whether SDIR1 gene from dicotyledon plant can also improve drought tolerance in monocotyledon plants, heading-stage Ubi-SDIR1 transgenic rice plants grown under water sufficient conditions were subjected to drought stress treatment (Fig. 3A). When water was withheld for 5 d, control rice plants exhibited visible drought stress symptoms, nearly all leaves rolling and leaf color turning pale green. In contrast, Ubi-SDIR1 transgenic rice plants with high SDIR1 levels were almost identical to non-stressed plants, except that older leaves exhibited slight rolling and were yellow (Fig. 3A). These results indicate that SDIR1 can improve drought tolerance in monocotyledon rice plants as well as dicotyledon plants.

It has been found that SDIR1 can enhance drought tolerance capacity by regulating stomata status in Arabidopsis. In this study, water loss assays of detached leaves were performed from unstressed tobacco (true leaves) and rice plants (corresponding to the third leaves from the end). After 2 h treatment, the fresh weight loss of detached leaves in 35S-SDIR1 transgenic tobacco was about 30%, corresponding to 40% for the control plants (Fig. 2C). Similar results were obtained for Ubi-SDIR1 transgenic rice plants (Fig. 3B). It perhaps SDIR1 overexpression can decrease water loss in plants. The smaller water loss in SDIR1 transgenic plants might be due to their stomata responding to water deficiency better than the control plants. Leaves from 35S-SDIR1 transgenic tobacco and control plants were examined for stomatal aperture status by the nail polish impression method. The stomatal apertures of 35S-SDIR1 transgenic tobacco plants were smaller than control plants (Fig. 2D). It was difficult for us to observe rice stomata status, and hence we present only the data for tobacco. These suggest that the stomata of 35S-SDIR1 transgenic tobacco plants responded to water deficit better than the control plants.

SDIR1 in transgenic tobacco and rice exhibited enhanced tolerance to drought as much as in Arabidopsis. This suggests that the SDIR1-ABA controlled process that determines the transpiration rate under water-deficit conditions and is conserved. SDIR1 in transgenic tobacco and rice plants may also play an active role in ABA-mediated guard cell control under drought conditions. ABA functions as a signal mediating adaptive responses, as in regulating downstream gene expression. It has been reported that Arabidopsis ABF3 constitutive expression in rice con-
Fig. 2. Response to Drought of 35S-SDIR1 Transgenic Tobacco Plants.

A. Drought tolerance assay of 8-week-old plants. Three-week-old plants were transplanted in soil in the same tray for another 5 weeks of growth; water was withheld by halting irrigation for drought treatment, and then they were rewatered. The photographs were taken 21 days after germination (21 DAG), 15 days (15 d) and 28 days (28 d) after drought, and 2 days (2 d) and 10 days (10 d) after rewatering. Representative plants from each treatment group are enlarged for better visualization. B. Survival rates of plants rewatered after 10 d, corresponding to (A). Percentages are means \( n = 9 \) to 24) of two repeats ± SD. Student’s t-test was used to test for statistical significance \( (P < 0.05) \) between the control line VC 1.3 and the transgenic lines. C. Transpiration rates. Leaves of the same developmental stages were excised and weighed at various time points after detachment. Each data point represents the mean of duplicate measurements. Error bars represent the mean of two independent experiments \( (n = 3) \). Values differed significantly from VC1.3 \( (P < 0.05) \). D. Measurement of stomatal aperture on 35S-SDIR1 transgenic tobacco and control lines. Stomatal guard cells were observed at beginning of the drought treatment by the nail polish impression method by microscopy. Data are mean ratios of pore width to length ± SE of two independent experiments \( (n = 40 \) to 60). Values differed significantly from VC1.3 \( (P < 0.05) \).

Fig. 3. Response to Drought of Ubi-SDIR1 Transgenic Rice Plants.

A. Drought tolerance assay of 90-d-old plants. Plants in equal-size containers were taken from the water field (top panel) for drought treatment for 5 d (bottom panel). Representative plants are shown. B. Transpiration rates. Leaves of the developmental stages corresponding to (A) were excised and weighed at various time points after detachment at room temperature. Each data point represents the mean of duplicate measurements. Error bars represent ± SE \( (n = 3) \). Values differed significantly from VC1.2 \( (P < 0.05) \).

SDIR1 Transgenic Rice and Tobacco Plant Drought Tolerance

SDIR1 overexpression causes drought tolerance in transgenic tobacco and rice plants. It can be assumed that SDIR1 overexpression in tobacco and rice plants regulates downstream ABA/stress-related genes in transgenic plants at least partially through ABF3 mediated ABA-dependent and independent pathways. Further functional investigation of the E3 ligase SDIR1, to search for its target proteins and other downstream signal components, is essential for a better understanding of ABA/stress signaling networks and for potential application in engineering plant stress tolerance.

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References


