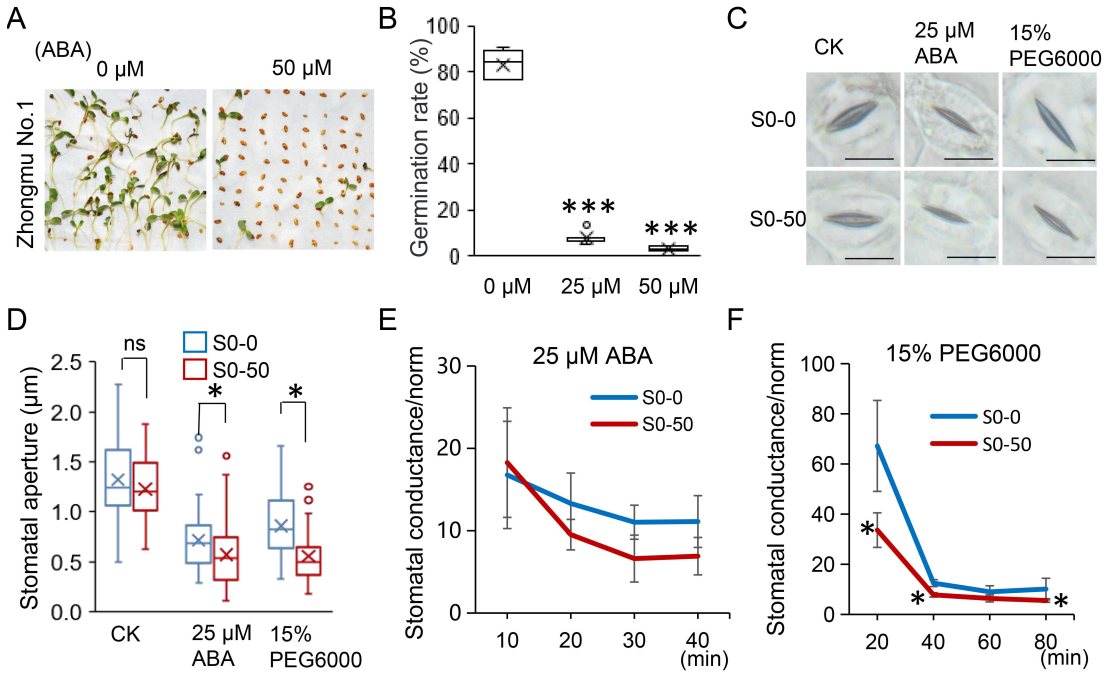

Fig. 1. Insensitve (S0-50) and sensitve (S0-0) alfalfa plants to ABA during seed germination and detached leaf stomatal movement assay.

(A) Germination of 'Zhongmu No.1' alfalfa seeds in the presence of 0μM or 50μM ABA. (B) Statistical analysis of germination rate in the presence of different concentration of ABA (n=3), (***P <0.001). (C) Photographs of stomata after 40 minutes of treatment with 0μM ABA (CK), 25μM ABA or 15% PEG6000. The scale bar measures 10 μm. (D) Statistical analysis of S0-0 and S0-50 stomatal aperture after 40min of treatment with 0μM ABA (CK), 25μM ABA or 15% PEG6000. At least 80 stomata from three separate experiments were measured. The data were shown as box plots with minimum to maximum plots. The center lines represent the medians, "×" represents the mean value, and the down and up whiskers extend to the minimum and maximum values. ns, no statistically significant difference; *P<0.05. (E, F) The relative stomatal conductance after 25μM ABA (E) or 15% PEG6000 (F) treatment compared to 0 min (as 100%). The data were presented as the means of three biological replicates (each with eight leaves) ± SD (*P <0.05).

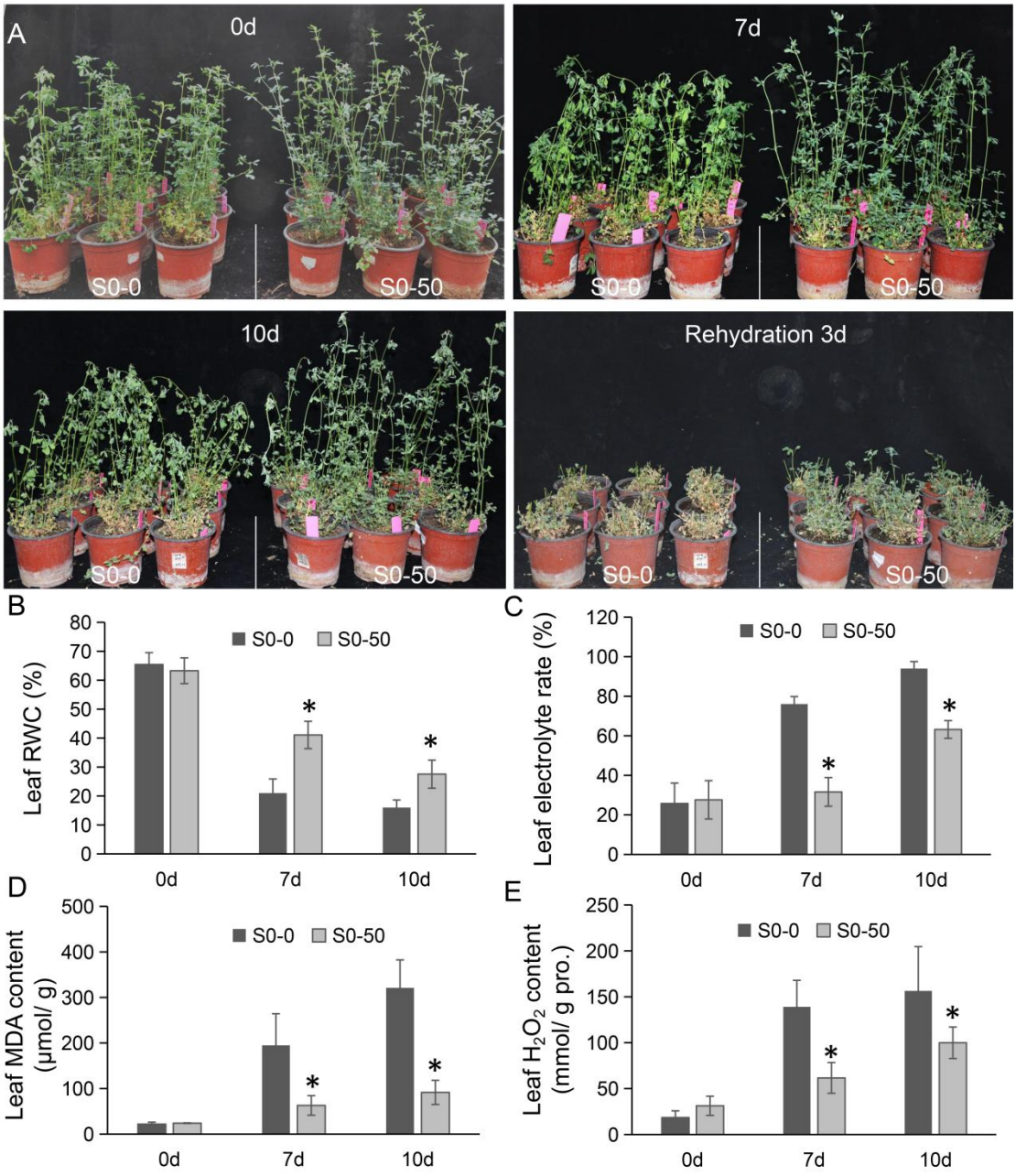

Fig. 2. S0-50 plant showed improved drought tolerance. (A) The phenotype of S0-0 and S0-50 plants before (0d) and after 7d, 10d for drought treatment and after rehydration for 3d. (B-E) Leaf relative water content (RWC) (B), electrolyte leakage (EL) (C), malondialdehyde (MDA) content (D) and leaf H_2O_2 content (E) before and after drought treatment. The data was shown as the mean of three biological replicates (with three technical repeats each) ± SD. An asterisk indicates statistically significant differences between S0-0 and S0-50 (P<0.05).

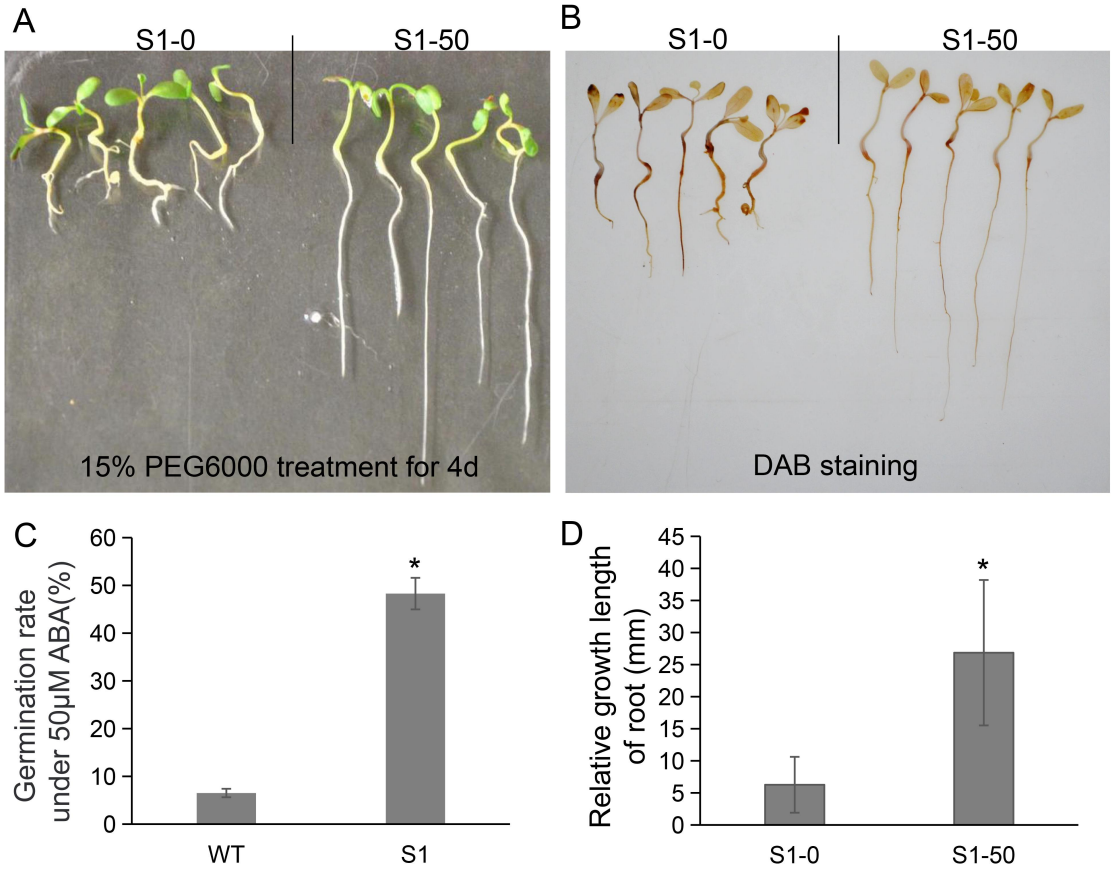

Fig. 3. Phenotype comparison of S1-0 and S1-50 alfalfa seedlings after PEG treatment. (A) Phenotype of S1-0 and S1-50 alfalfa seedlings after 4 days of 15% PEG6000 treatment. (B) H_2O_2 content test using diaminobenzine (DAB) staining of alfalfa seedlings after 4 days of 15% PEG6000 treatment; a darker brown indicates a higher H_2O_2 content. (C) Germination rate of alfalfa wild type (WT) seeds and S1-50 alfalfa seeds treatment with 50 μM ABA. (D) The relative growth length of seedling roots after 4 days for 15% PEG6000 treatment. The data was shown as the mean of three biological replicates (with three technical repeats each) ± SD. An asterisk indicates statistically significant difference between S0-0 and S0-50 (P<0.05).

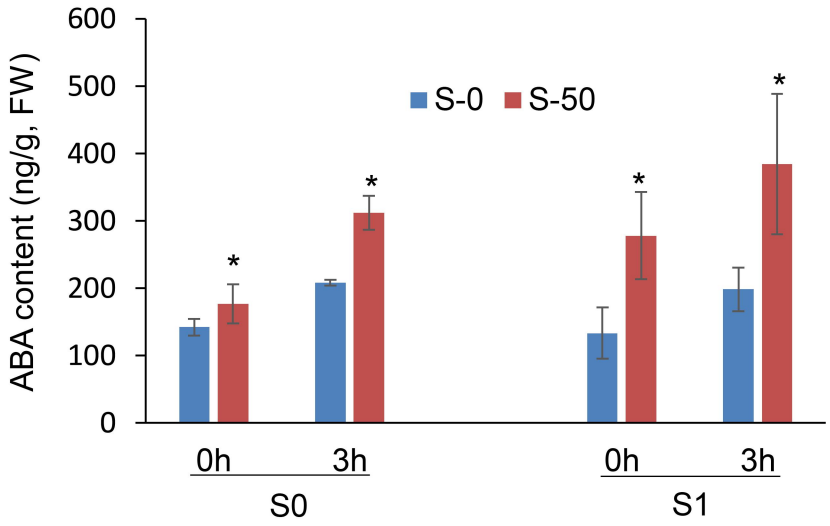

Fig. 4. ABA content of four-month-old S0 plant leaves and seven-day-old S1 seedlings before and after 15% PEG6000 treatment. The data was shown as the mean of three biological replicates (with three technical repeats each) ± SD. The asterisk indicates statistically significant differences between S0-0 and S0-50 (P<0.05).

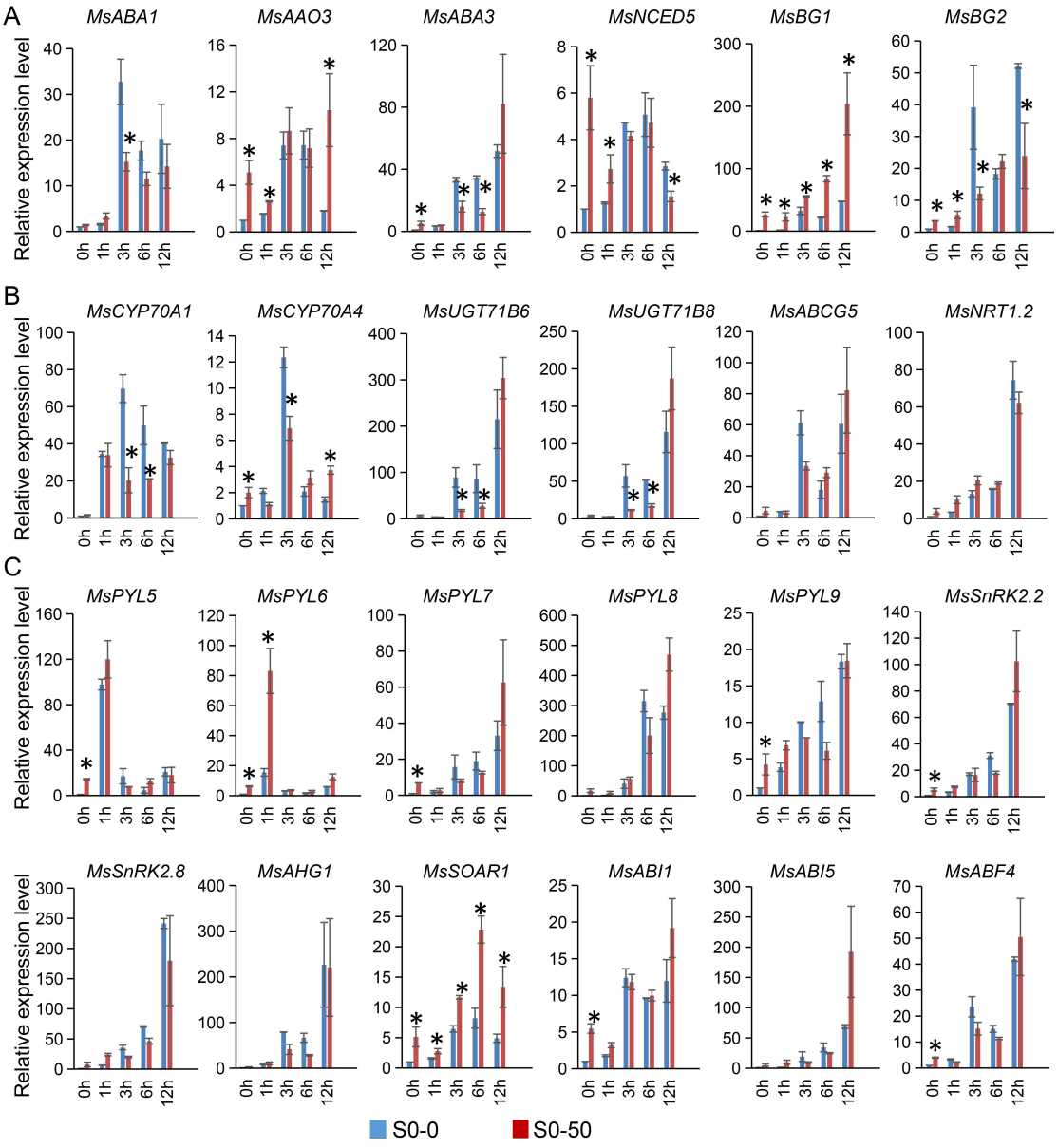

Fig. 5. Gene expression tests of second internode leaves from four-month-old S0-0 and S0-50 alfalfa after PEG treatment. (A) Gene expression of ABA biosynthesis-related genes, (B) ABA catabolism and long-distance transport, and (C) ABA signaling transduction-related genes before and after 15% PEG6000 treatment. The data was shown as the mean of three biological replicates ± SD. The asterisk indicates statistically significant differences between S0-0 and S0-50 (P<0.05).

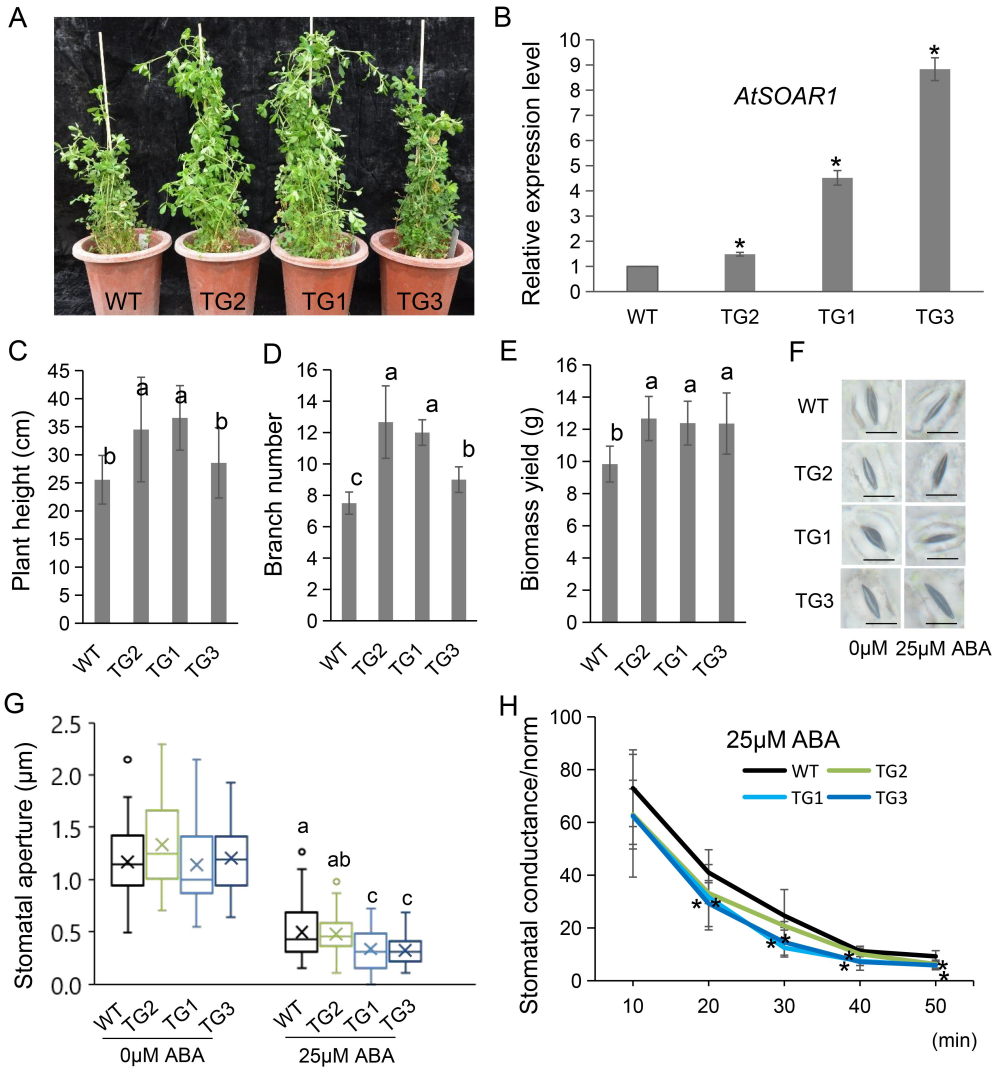

Fig. 6. Overexpressing *AtSOAR1* reduced alfalfa ABA sensitivity. (A) Phenotype photograph of 4 months old wild type (WT) and *AtSOAR1* transgenic (TG) alfalfa plants. (B) Relative expression level of *AtSOAR1* in WT and TG plants. Error bars, SD (n=3). *P < 0.05. (C-E) Plant height (C), branch number (D), and up-ground biomass yield (E) of four-month-old WT and TG plants. The data showed as the mean of three biological replicates ± SD. The letters indicate the significant difference (P < 0.05). (F) Photographs showed the stomata after treatment with 0 μM ABA or 25μM ABA for 40 min. The scale bar represents 10μm. (G) Statistical analysis of stomatal aperture in WT and TG plants after 40 minutes for 0 μM ABA or 25μM ABA treatment. At least 80 stomata from three separate experiments were measured. The center lines show the medians, "×" shows the mean value, and the down and up whiskers extend to the minimum and maximum values. The different letters indicate the significant differences (P < 0.05). (H) The stomatal conductance analysis in detached leaves from WT and TGs at various time points after 25μM ABA treatment. The data were shown as the means of three biological replicates (each with eight leaves) ± SD (*P <0.05).

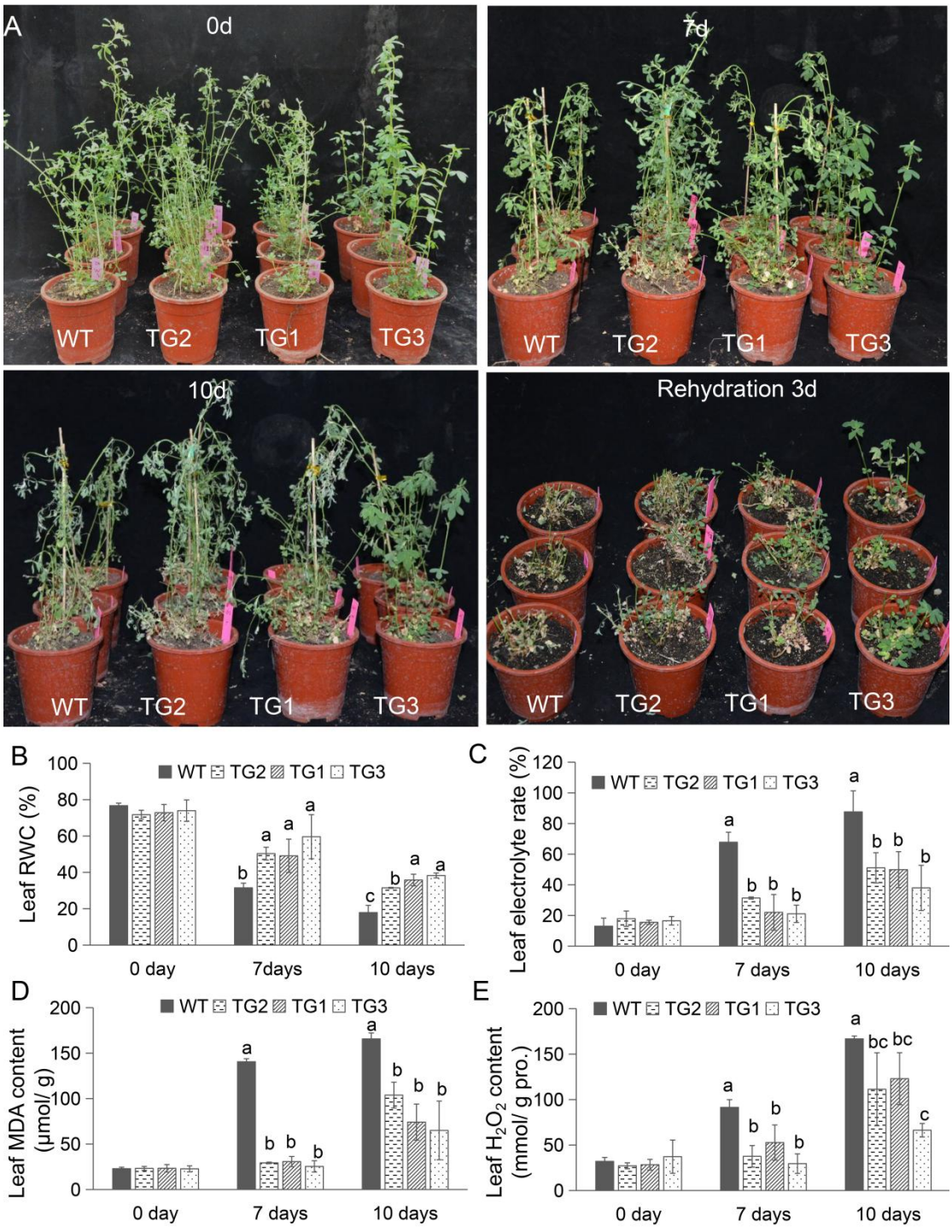

Fig. 7. Overexpression of *AtSOAR1* enhanced drought tolerance of alfalfa transgenic plants (TGs). (A) Phenotype of WT and TG plants before (0d) and after drought treatment with water control for 7d, 10d, and rehydration (3d). (B-E) Leaf RWC content (B), EL (C), MDA content (D), and leaf H_2O_2 content (E) before and after drought treatment. The data was shown as the mean of three biological replicates ± SD. The different letters indicate statistically significant differences determined by Duncan's multiple range test ($P < 0.05$).

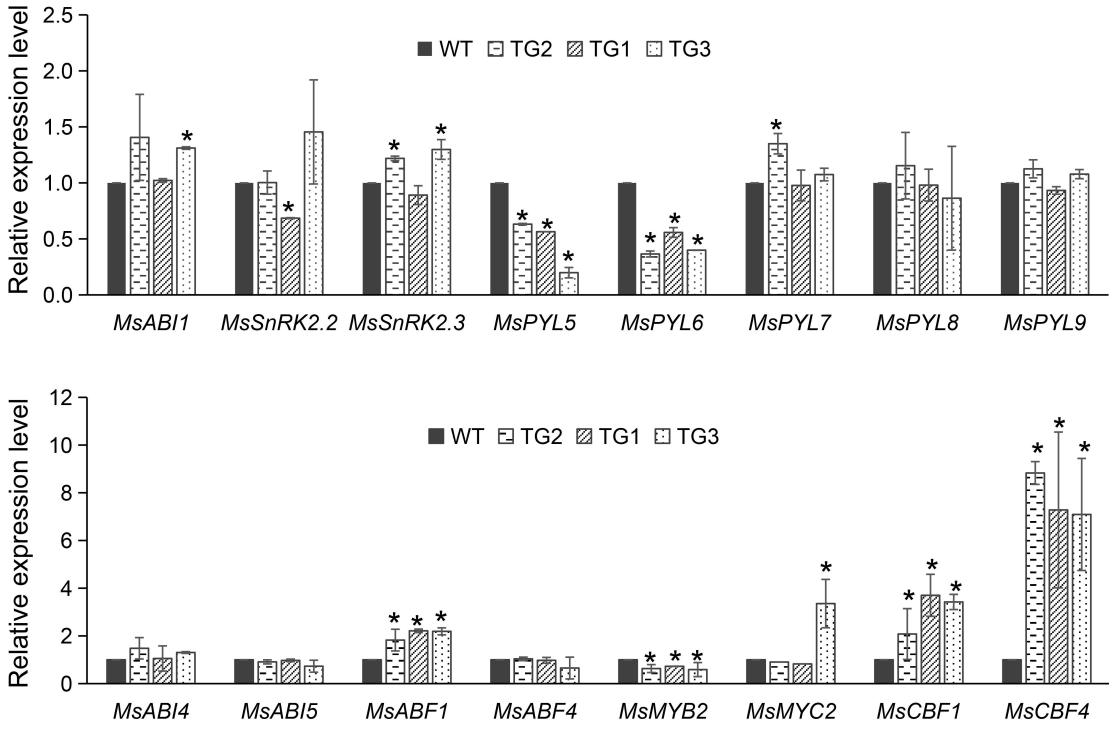

Fig. 8. Expression of ABA-dependent and ABA-independent drought response genes in WT and *AtSOAR1* alfalfa transgenic plants. The data was shown as the mean of three biological replicates ± SD. An asterisk indicates statistically significant differences between WT and TG (P<0.05).