P-44: The role of ABA-responsive element binding factors in proline biosynthesis in Arabidopsis and barley

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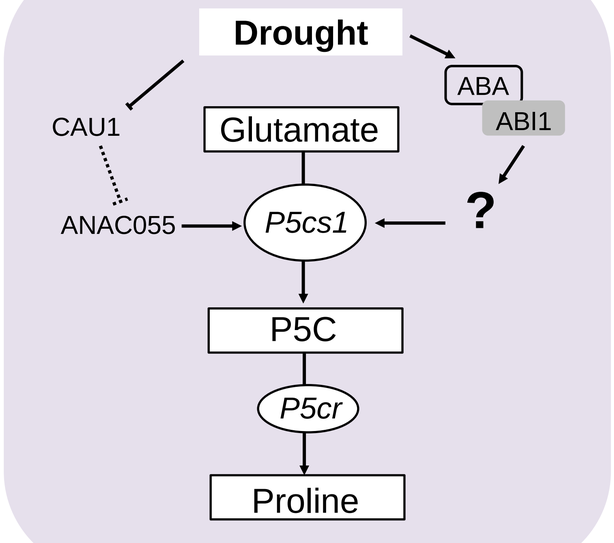
Background

Proline accumulation is one of the apparent responses of plants against drought stress. The primary role of proline is to maintain membrane stability and protect macromolecule structure during osmotic stress. Pyrroline-5-carboxylate synthase 1 (*P5cs1*) catalyzes the rate-limiting step of reduction of glutamate to proline under osmotic stress. Therefore, transcriptional upregulation of *P5cs1* is considered as a hallmark for cytosolic proline deposition under drought stress conditions. Drought stress responses are generally divided in ABA-dependent and ABA-independent signaling pathways. ABA-responsive elements (ABREs) are critical regulatory elements found in the promoter of osmotic stress-responsive genes and are the direct target of ABRE binding factors (AREBs/ABFs). Although ABF transcription factors are fundamental components of ABA-driven transcription cascades, their role in modulating proline accumulation remains enigmatic. Therefore, the present study aims to dissect the critical role of AREBs/ABFs in proline biosynthesis under different water stress scenarios in the model plant Arabidopsis. The findings from Arabidopsis were used to identify similar processes in barley.

Research question



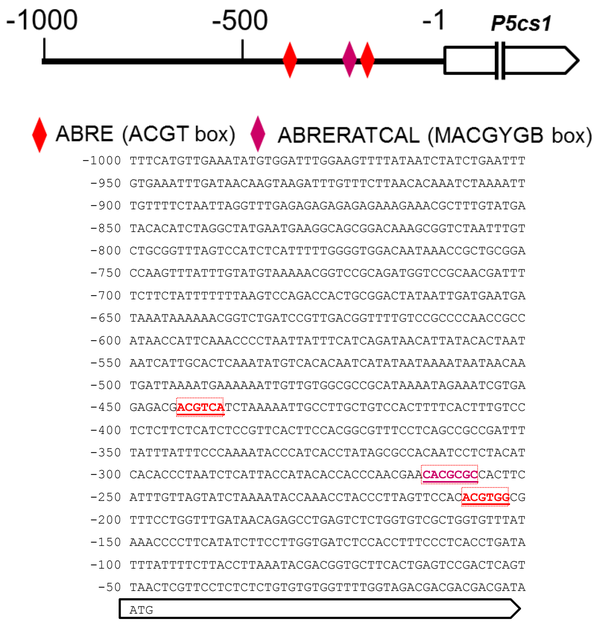


Figure 1. Position of ABRE motifs in *AtP5cs1* promoter predicted by PLACE and PLANT PAN database.

Results

1. ABFs are involved in proline accumulation upon ABA treatment

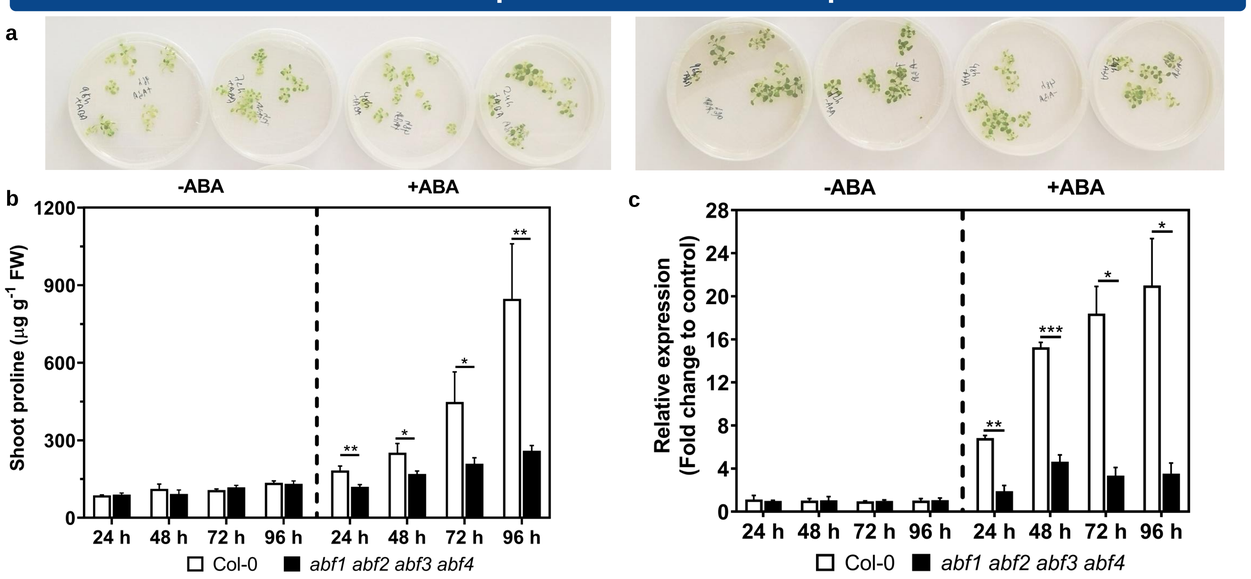


Figure 2. Proline accumulation and *AtP5cs1* expression in response to external ABA application. (a) Experimental setup. (b) Shoot proline concentration in Arabidopsis after 24, 48, 72 and 96 h of ABA (50 µM) treatment. Before sampling, two week old seedlings were cultivated on MS medium with and without ABA. The graph represents the mean ± SE (n = 8). (c) Relative mRNA expression of *AtP5cs1* after 24, 48, 72 and 96 h of ABA treatment. The graph represents the mean ± SE (n = 3). Asterisks indicate significant differences between genotypes (P* < 0.05, ***P* < 0.01, ****P* < 0.001) using student's *t*-test. FW, fresh weight**

2. Proline determination and *P5cs1* expression under acute dehydration

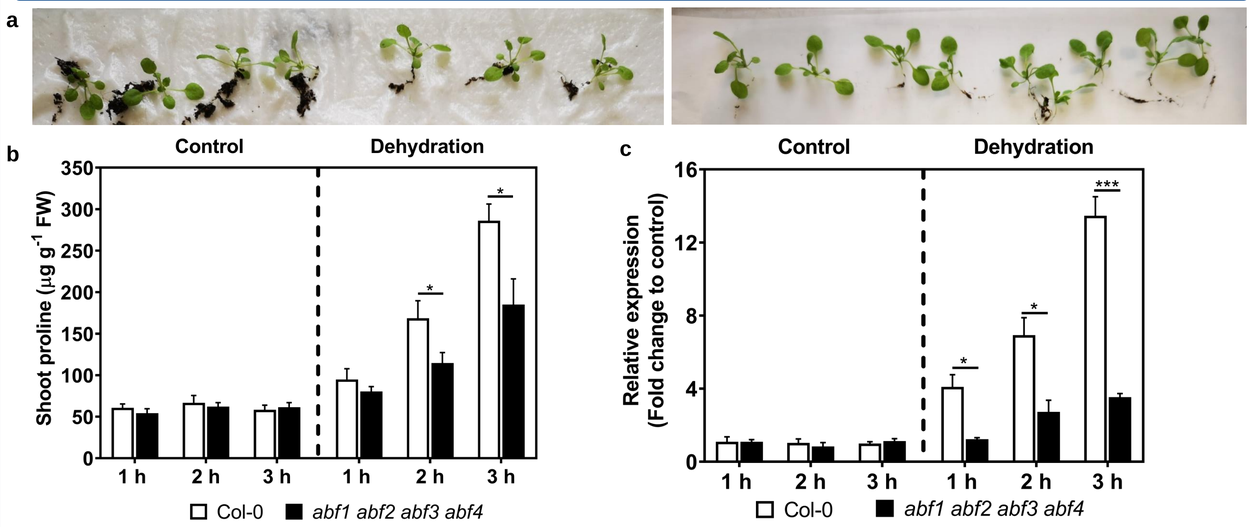


Figure 3. Proline accumulation and *AtP5cs1* expression in response to acute dehydration. (a) Shoot proline concentration in Arabidopsis at 1, 2 and 3 h of acute dehydration. Fifteen days old seedlings were removed from the soil and placed over parafilm after washing the roots. The graph represents mean ± SE (n = 6). Asterisks indicate significant differences between genotypes (P* < 0.05) using student's *t*-test. (b) *P5cs1* expression in response to acute dehydration. Relative mRNA expression of *AtP5cs1* after 1, 2 and 3 h of acute dehydration. The graph represents mean ± SE (n = 3). Asterisks indicate significant differences between genotypes (**P*<0.05, ***P* < 0.01, ****P* < 0.001) using student's *t*-test. FW, fresh weight**

3. Plant growth and proline accumulation under drought

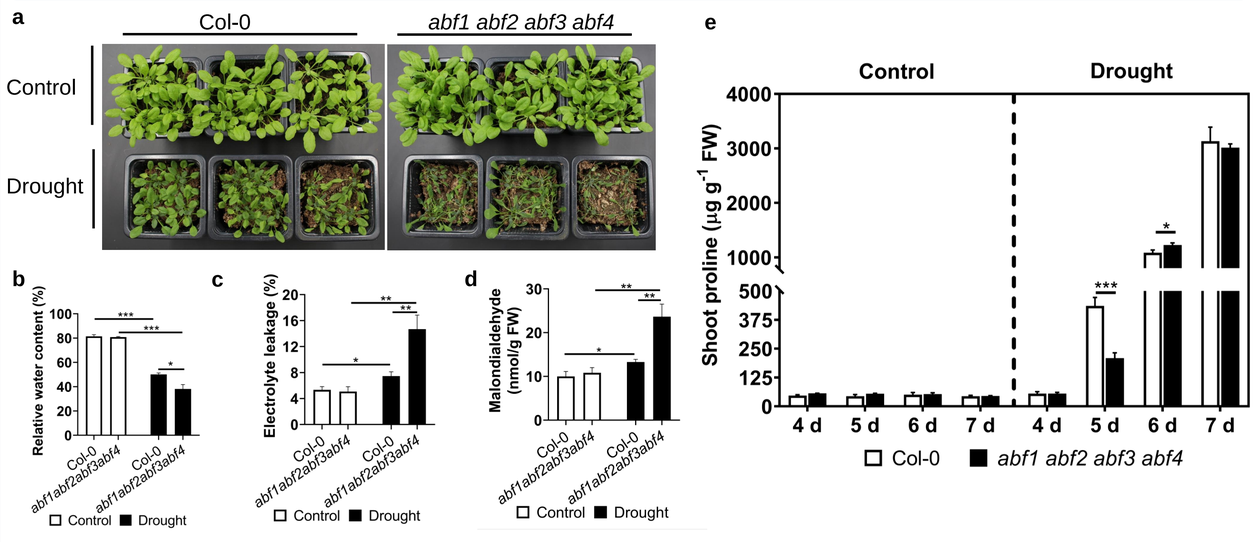


Figure 4. Morphological and physiological responses to drought. (a) Effect of drought on the growth of wild type and the *abf1 abf2 abf3 abf4* quadruple mutant. Terminal drought was applied to 21 days old seedlings. Pictures were taken 7 d after the drought. The effect of drought on (b) Relative water content (c) Electrolyte leakage (d) Malondialdehyde concentration. The analyses were done in samples collected at 7 d after drought (e) Shoot proline concentration at 4, 5, 6 and 7 d after drought. Bar indicates mean ± standard error (n = 6). Asterisks indicate significant differences between genotypes (P* < 0.05, ***P* < 0.01, ****P* < 0.001) using student's *t*-test. FW, fresh weight**

4. Allele mining identified novel promoter alleles in barley diversity set

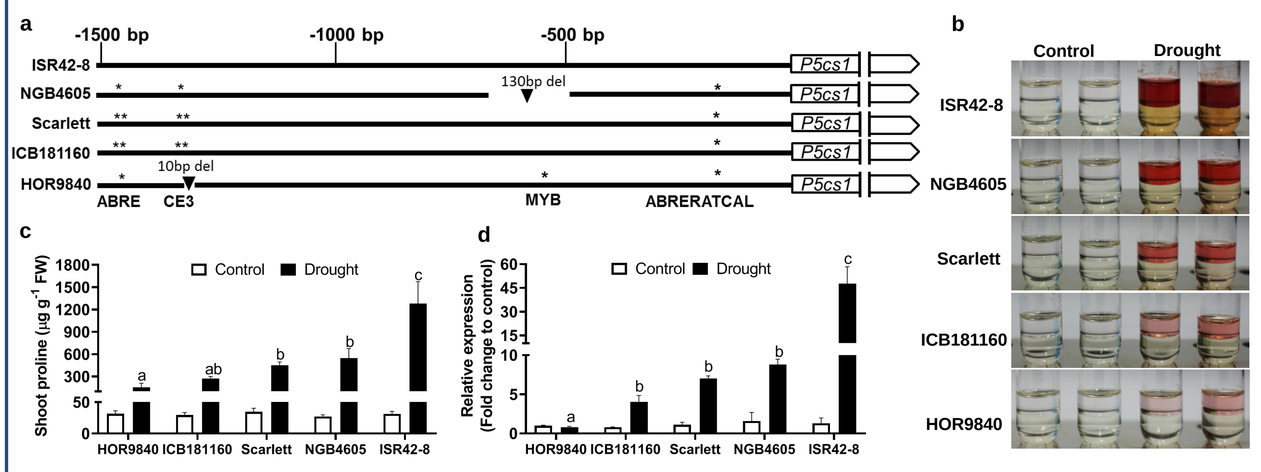


Figure 5. Allele mining of barley *HvP5cs1* promoter. Sixty barley comprised of wild accessions, landraces and cultivars, were sequenced. (a) Schematic diagram of *HvP5cs1* promoter region. Five *HvP5cs1* promoter haplotypes based on polymorphisms across the DNA binding motifs were detected. Asterisks indicate SNP and triangles indicate deletion. (b) Free proline detection from shoot extracts using Ninhydrin reagent. A darker color indicates higher proline concentration in the shoot samples. Two week old seedlings were exposed to nine days of drought before proline measurement. (c) Shoot proline concentration of *HvP5cs1* promoter haplotypes. Indexed letters above the bars indicate significant differences between the genotypes (*P* < 0.05) not sharing the same letter under drought condition. Bars represent mean ± SE (n = 8). (d) Relative mRNA expression of the barley *HvP5cs1* gene. Indexed letters above the bars indicate significant differences between the genotypes (*P* < 0.05) not sharing the same letter under drought condition. Bars represent mean ± SE (n = 3). FW, fresh weight

5. Targeting barley ABFs (HvABFs) with CRISPR RNA/Cas9 system

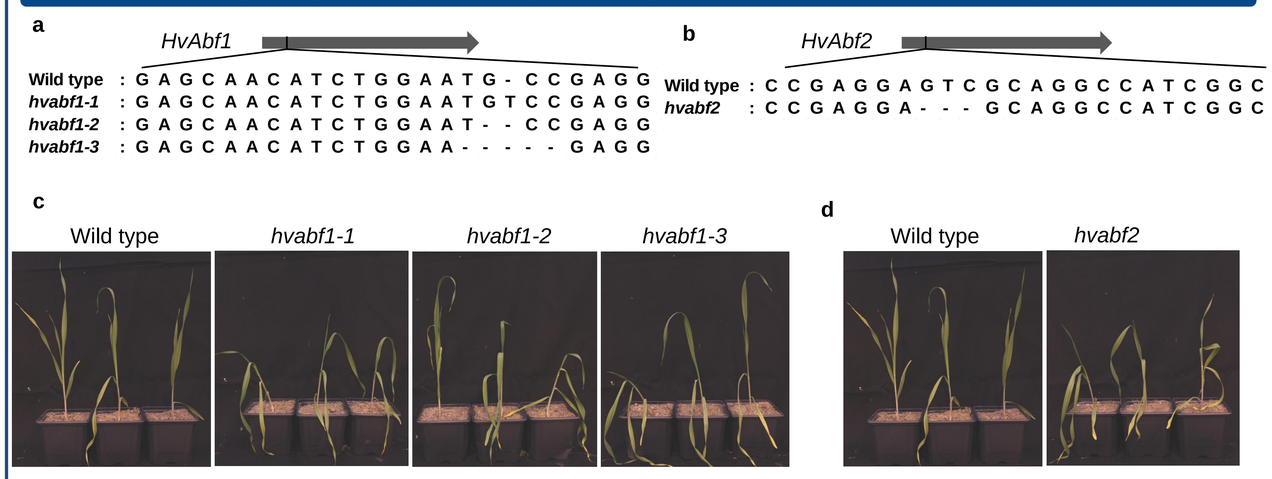


Figure 6. Sequence analysis of target sites of putative barley ABFs (T2 plants) (a) The mutation events in HvABF1 with one bp insertion (*hvabf1-1*), one bp deletion (*hvabf1-2*) and four bp deletion (*hvabf1-3*). These mutations resulted in gene knock-out owing to translational frameshift. (b) The mutation event in HvABF2 with three bp deletion (*hvabf2*). The mutation resulted in deletion of serine in a conserved domain of HvABF2. The phenotype of wild type, (c) *hvabf1* mutants and (d) *hvabf2* mutants under drought. Two week old plants were exposed to terminal drought by withholding watering. The pictures were taken one week after drought treatment.

Conclusion and Outlook

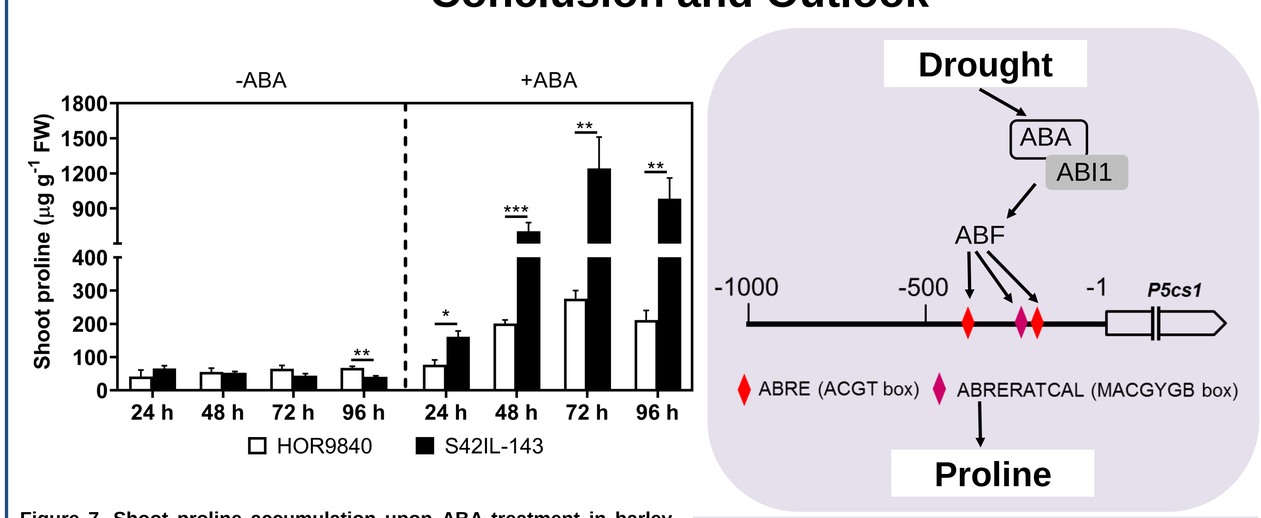


Figure 7. Shoot proline accumulation upon ABA treatment in barley. One week old seedlings were cultivated in hydroponics with half-strength Hoagland's solution for 7 d. Then, seedlings were transferred to a fresh Hoagland's solution supplemented with and without ABA (50 µM). Samples were collected at 24, 48, 72 and 96 h of ABA treatment for proline measurement. Bar indicates mean ± standard error (n = 4). Two plants were pooled together as one biological replicate. Asterisks indicate significant differences between genotypes (P* < 0.05, ***P* < 0.01, ****P* < 0.001) using student's *t*-test. S42IL-143 is an introgression line in Scarlett background carrying QTL allele from ISR42-8 controlling drought-inducible proline accumulation. FW, fresh weight**

In barley, we detected natural variation in *HvP5cs1* **promoter across ABA-responsive elements,** related **coupling elements** and **MYB** motifs. It also showed ABA responsiveness to ABA application. The ongoing work on barley involves:

- Screening of HvABF knock-out mutants for proline accumulation at different drought scenarios.
- *In vitro* DNA-protein binding assay (EMSA) to prove that HvABFs target ABRE motifs of barley *HvP5cs1* promoter.

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