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Overexpression of *Arabidopsis* H^+-pyrophosphatase improves the growth of alfalfa under long-term salinity, drought conditions and phosphate deficiency

Jia-Hao Su, Tian-Hui Bai, Fei Wang, Ai-Ke Bao

State Key Laboratory of Grassland Agro-ecosystems, Key Laboratory of Grassland Livestock Industry Innovation, Ministry of Agriculture and Rural Affairs; College of Pastoral Agriculture Science and Technology, Lanzhou University, Lanzhou , P.R. China

Electronic Supplementary Material (ESM)

Supplementary Information

Vector and transformation procedure

***Agrobacterium* strain and vector**. *Agrobacterium tumefaciens* strain GV3101 containing pRG395 vector was used for transformation. The pRG395 vector was kindly granted by Prof R. Gaxiola from Arizona State University and carries the coding sequence of the *AVP1* gene under control of a tandem repeat of the CaMV35S promoter with the *nptII* (neomycin phosphotransferase II) gene conferring resistance to kanamycin as previously described by Park *et al.* (*PNAS*, 2005, 102, 18830–18835.).

Generation of transgenic alfalfa. The hypocotyls from four-day-old seedlings of alfalfa (*M. sativa* L. *cv* Xinjiang Daye) as explants were used for transformation. *A. tumefaciens* containing the plasmid was cultured in liquid LB medium supplemented with 75 mg/l kanamycin for 2 days at 180 rpm, 28°C. The bacterial suspension was centrifuged at 4000 rpm for 10 min at 4°C and re-suspended in liquid MSH medium (MS medium supplemented with 5.0 mg/l 2, 4-D and 1.0 mg/l kinetin) to the final concentrations (OD_{600} = 0.5–0.7). Meanwhile, the hypocotyl explants were firstly pre-cultured on MSH medium for 4 days in the dark at 24°C, then they were immersed into bacterial suspensions for 20 min. Treated explants were blotted with sterilized filter paper and co-cultivated on MSH medium for 4 days under dark at 24°C. After co-cultivation, the infected explants were washed with sterilized ddH_2O containing 200 mg/l carbenicillin, then they were cultured to MSH medium containing 200 mg/l carbenicillin in the dark at 24°C. The embryogenic calluses were transplanted onto SH medium containing 75 mg/l kanamycin and 100 mg/l carbenicillin for 8 weeks. To screen the transformants, the surviving resistant somatic embryos were cultured on half-strength MS medium supplemented with 10 g/l sucrose and 40 mg/l kanamycin at 24°C for 4 weeks. Finally, the rooting resistant plants were transferred into plastic pots (cylindrical pots of 8 cm in diameter and 10 cm in height, 1 plant/pot) containing the artificial soil with a mixture of vermiculite, perlite, and peat moss (v/v, 1: 1:1) and cultured in the greenhouse under a photoperiod 16 h/8 h (light/dark) at 28°C in the light and 24°C in the dark. The wild-type (WT) plants were obtained via the same tissue culture process used to produce transgenic plants (without transformation and selection).

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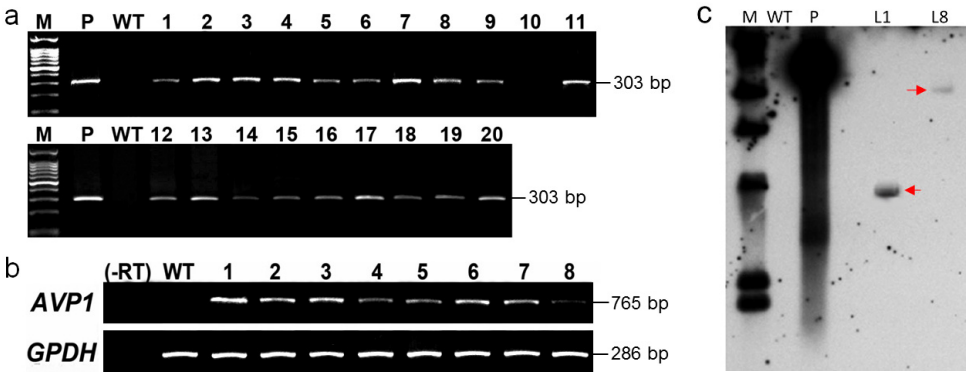

Figure S1. Molecular characterization of transformed alfalfa plants: genomic DNA PCR analysis of wild-type (WT) plants and transgenic lines (1-20) using *AVP1* specific primers (a), expression analysis of *AVP1* by RT-PCR in random 8 transgenic alfalfa lines (1–8), and alfalfa GPDH gene fragment was amplified as an internal control (b), southern blot analysis showed that *AVP1* was integrated into the genome of transgenic alfalfa lines L1 and L8 with single copy (c)
M – mark; P – plasmid DNA carrying *AVP1*; (-RT) – without reverse transcriptase

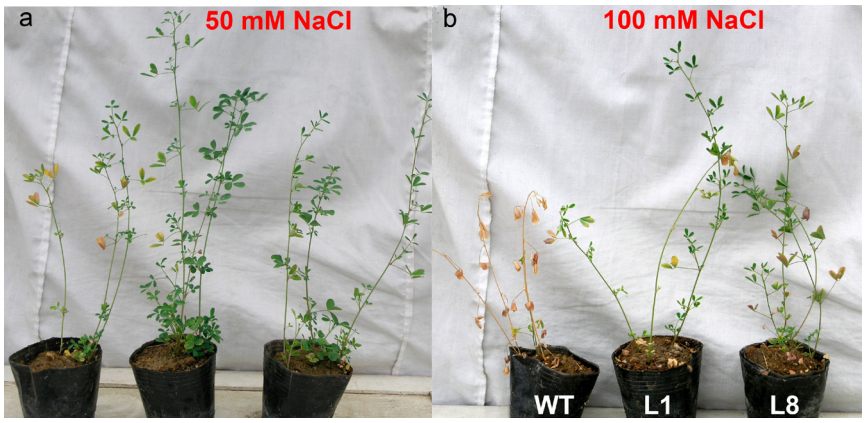

Figure S2. Growth of wild-type plants and transgenic alfalfa under long-term salt treatments; the photographs show the representative plants that were treated for 20 days with 50 mM (a) and 200 mM (b) NaCl
WT – wild-type plants; L1, L8 – transgenic lines overexpressing *AVP1*

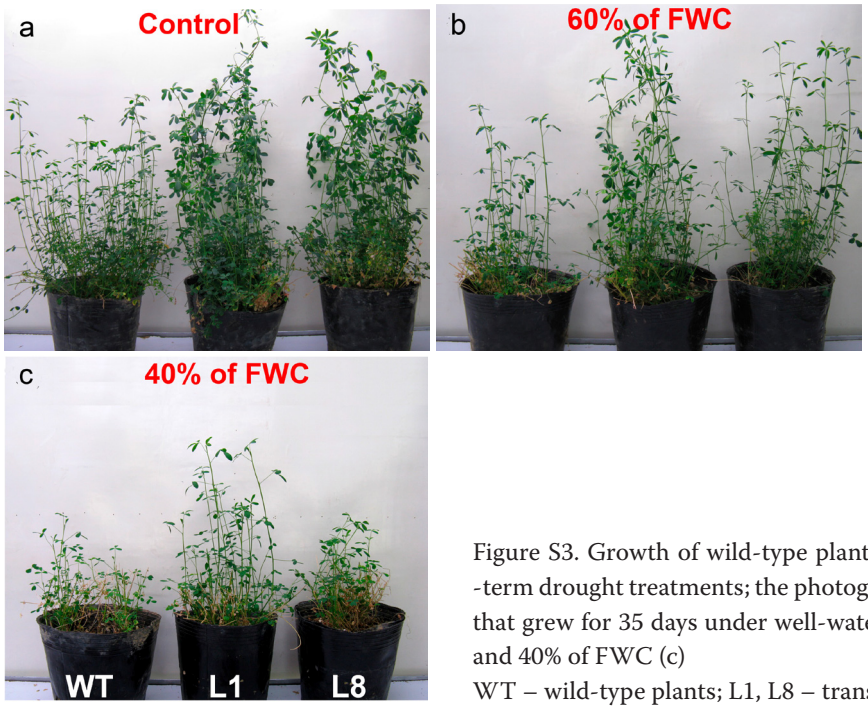

Figure S3. Growth of wild-type plants and transgenic alfalfa under long-term drought treatments; the photographs show the representative plants that grew for 35 days under well-watered conditions (a), 60% of FWC (b) and 40% of FWC (c)
WT – wild-type plants; L1, L8 – transgenic lines overexpressing *AVP1*

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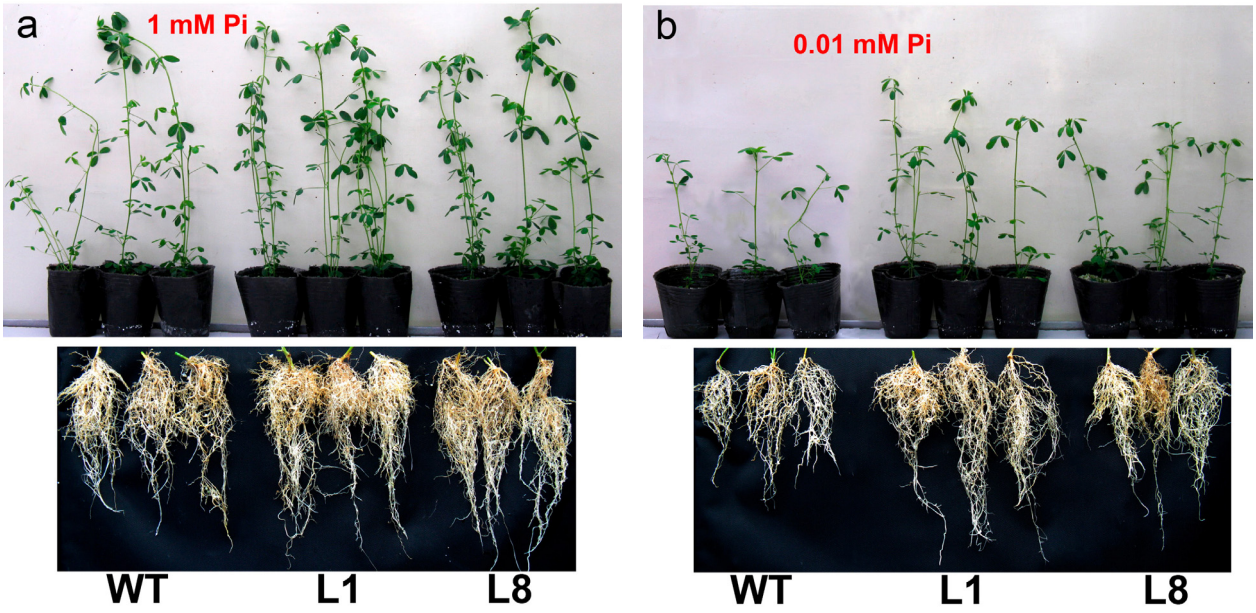

Figure S4. Growth of wild-type plants and transgenic alfalfa under phosphate deficiency (low-Pi) conditions; the photographs show the representative plants that were treated for 21 days with (a) 1 mM Pi (control) and (b) 0.01 mM Pi (low-Pi)
WT – wild-type plants; L1, L8 – transgenic lines overexpressing *AVP1*