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Overexpression of the *Arabidopsis* AVPI gene enhanced the salt-and drought-tolerance in transgenic alfalfa (*Medicago sativa* L.)

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Key words: H⁺-PPase, AVPI gene, transgenic alfalfa, salt-and drought-tolerance

Introduction there have been not any reports about improving the salt and drought tolerance of alfalfa by introducing AVPI. We assume that transgenic alfalfas expressing the H⁺-PPase gene are indeed more resistant than wild-type plants to salt stress and soil water deficit. This assumption is to be testified in this study.

Materials and methods Hypocotyls (2-4 mm) cut from 4-day-old seedlings were used as explants for transformation mediated by *Agrobacterium* strain GV3101 carried the AVPI gene which was granted by Gaxiola in 2004. The transgenic plants were identified by PCR method. The expression of AVPI in transformants was determined using RT-PCR. The cation concentrations were measured using a flame spectrophotometer. The solute potential was determined with a cryoscopic osmometer. The net photosynthetic rate was measured using an automatic photosynthetic measuring apparatus. The MDA content was determined using thiobarbituric acid (TBA) protocol. The relative membrane permeability of leaf cells was determined using a conductivity meter.

Results A high efficiency *Agrobacterium*-mediated transformation system in alfalfa was established. This system showed higher transformation efficiency (about 2.1%) and a shorter time (about 18-19 weeks) compared with most of previous procedures for alfalfa. By using this system, 20 Kan^R plants were obtained in this study. Expected PCR products (about 303 bp) were obtained from 19 lines of 20 Kan^R lines, suggesting that the AVPI gene was integrated into the chromosomal DNA of these alfalfa plants (Figure 1A). To measure the expression level of AVPI mRNA, RT-PCR was performed on random eight transgenic lines together with wild-type plants. All lines expressing the AVPI gene and AVPI mRNA levels varied among individual transformants (Figure 1B), and line 1 (highest) and line 8 (lowest) were chosen for further physiological assays. Transgenic alfalfa grew well in the presence of 200 mM NaCl (Figure 2) and in the environment of withholding water and rewatering (Figure 3A, B), while wild-type plants exhibited wilting and growth inhibition, even death. Compared with wild-type plants, transgenic plants accumulated more Na⁺, K⁺ and Ca²⁺ in leaves and roots.

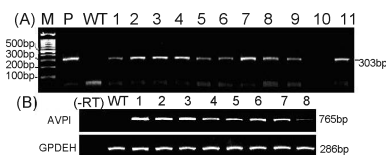


Figure 1 Molecular detection of alfalfa transformants. (A) PCR analysis; (B) RT-PCR analysis.

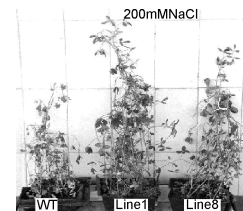


Figure 2 Transgenic alfalfa plants showed enhanced salt tolerance. Plants of wild type and two transgenic lines at 200 mM NaCl for 10 d.

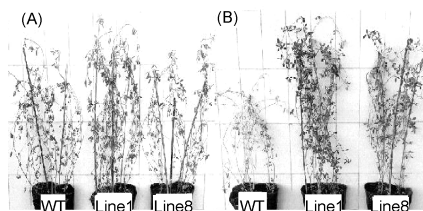


Figure 3 Transgenic alfalfa plants showed enhanced drought tolerance. (A) The well water soil was allowed to dry by withholding water for 8 d. (B) Then soil was rewatered to field capacity for 4 d.

Conclusions The overexpression of *Arabidopsis* H⁺-PPase gene confers salt-and drought-tolerance in alfalfa. This is associated with the increased sequestration of Na⁺ into vacuole, which resulted in the overexpression of H⁺-PPase gene and led to accumulation of solutes and maintaining ion homeostasis especially intracellular K⁺ and Na⁺ homeostasis.