Overexpression of the *Arabidopsis AVP* 1 Gene Enhanced the Salt- and Drought-Tolerance Intransgenic Alfalfa (*Medicago sativa* L.)

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Overexpression of the *Arabidopsis AVP*1 gene enhanced the salt-and drought-tolerance in transgenic alfalfa (*Medicago sativa* L .)

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Key words : H^+-PPase ,*AVP1* 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 *AVP1* . 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 *AVP1* gene which was granted by Gaxiola in 2004 . The transgenic plants were identified by PCR method . The expression of *AVP1* 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 *AVP1* gene was integrated into the chromosomal DNA of these alfalfa plants (Figure 1A) . To measure the expression level of *AVP1* mRNA , RT-PCR was performed on random eight transgenic lines together with wild-type plants . All lines expressing the *AVP1* gene and , *AVP1* 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 Ca^{2+} in leaves and roots .

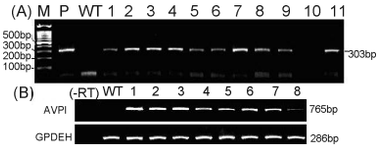

Figure 1 *Molecular detection of alfalfa transformants . (A) PCR analysis ; (B) RT-PCRanalysis .*

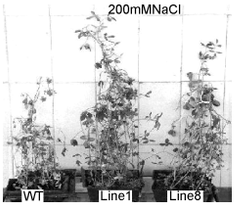

Figure 2 *Transgenic alfalfa plants showed enhanced salt tolerance . Plants of wild type and two transgenic lines at 200 mM NaCl for 10 d .*

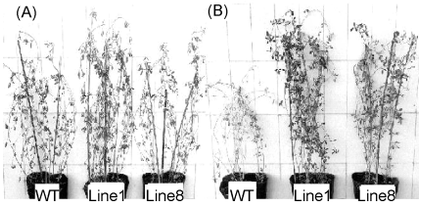

Figure 3 *Transgenic alfalfa plants showed enhanced drought tolerance . (A) The well water soil was allowed to dry by withholding water for 8 d . (B) Then soil was rewatered to field capacity for 4 d .*

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 .