Cloning and expression analysis of the vecoule membrane Na^+/H^+ antipoter gene in *Medicago stativ L.*

Yang Qing-chuan Wu Ming-sheng Wang Ping-qing Hou Xiang-yang

Institute of Animal Science, Chinese Academy of Agricultural Sciences,
Beijing 100094, China

Vacuolar compartmentation of Na^+ through Na^+/H^+ antiporters in the large vacuole membrane is an essential mechanism for salinity tolerance of plants. First, sequestration of Na^+ into vacuole could lowers cytosolic Na^+ level, which keep redundant Na^+ form the sites of metabolism and alleviate the toxic effects to enzymes and membrane system; Second, plant could take advantage of enough Na^+ in vacuole to lower the water potential of cell and to resist to osmotic stress of salt. Moreover, efficient Na^+ uptake into vacuole could maintain the high cytosolic K^+/Na^+ rate.

In this study, by the multiply alignment of amino acid sequences and nucleotide acid sequences of several plant vacuole membrane Na^+/H^+ ant porters in embank, a pair of degenerate primers were designed in the conserved regions that were used for RT-PCR to amplify a 288bp coda segment from the medic ago sativa. Base on the sequence of the cDNA segment , primer was designed for 3' RACE and a 1536bp coda segment was acquired; from the sequence of 3' end of the gene, primer was designed for 5'RACE and a 1082bp cDNA segment was acquired. The full-length cDNA was gained by overlapping sequences and the analysis of the sequence indicated it contained a open reading frame, comprising 555 amino acid residues. The amino acid sequence compared by Blast revealed high homology with that of other plant vacuole membrane Na^+/H^+ antiporters, the similarity to InNHX1 was highest with 78%. The result indicated the gene cloned form alfalfa is a vacuole membrane Na^+/H^+ antiporter gene, which we have named *MsNHX1*. The Genbank accession number of it is BAB49004.

RT-PCR was performed to reveal transcript level of *MsNHX1* in different tissues and under different abiotic stresses. The results indicated *MsNHX1* is abundant in leaf and stem, rather than in root; Furthermore, the transcript level of *MsNHX1* was up-regulate by 200 mM NaCl and reached its peak after 6 hour , but not by drought and cold treatment.

Kyewords: *Medicago sativa* L., Vacuole Membrane, Na^+/H^+ Antiporter, RT-PCR,