Molecular Clone of the Na^+/H^+ Antiporter Gene *AtNHX1* and Study of Transgenic Salt Tolerant Lucerne

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Molecular clone of the Na^+/H^+antiporter gene *AtNHX1* and study of transgenic salt tolerant lucerne

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Introduction Lucerne (*Medicago sativa*) with its good quality and ease of cultivation occupies an important position in animal feeding. Salinity is a major constraint of crop productivity, because it reduces yield and limits expansion of agriculture. Na^+/H^+ antiporter catalyses the counter transport of Na^+ and H^+ across membranes. Vacuolar Na^+/H^+ antiporter plays an important role in developing salt-tolerance of plants. Therefore, we could use the gene involved in this mechanism to modify salt tolerance of lucerne.

Materials and methods This experiment in molecular biology is based on Gene Bank data. We designed a pair of primers and used reverse-transcription PCR to isolate a DNA fragment of 1.6kb in length from *Arabidopsis thaliana* (Columbia type). The target fragment was ligated into pGEM-T easy vector and subject to DNA sequencing. It was introduced into lucerne by *Agrobacterium*-mediated transformation.

Results DNA sequencing with the target fragment shows that it contains a whole open reading frame. We selected a fragment of *AtNHX1* encoding peptide rich in hydrophilic residues and designed primers. We induced callus of seedling cotyledon and hypocotyls of lucerne. A plant expression vector harbouring the *AtNHX1* gene was constructed and introduced into lucerne by *Agrobacterium*-mediated transformation. The promoter used in our experiment was CaMV 35S. Three cultivars of lucerne were used for the transgenic experiment. Regenerated plants were subjected to genome-PCR and Southern blotting to attest the stability of *McNHX1* in acquired salt tolerant lucerne plants. Based on the data from EST of Crystalline Iceplant (*Mesembryanthemum crystallinum*) to obtain *McNHX1* from vacuole membrane by RACE and RT-PCR, we will introduce it into lucerne to get a more salt resistant cultivar and apply a heterologous Na^+/H^+ antiporter gene to improve salt tolerance of other agricultural crops.

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