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Genetic engineering of salt tolerant plants

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Accumulation of salts in irrigated soil are primary factors depressing yield in crop production, because the major crops are almost universally non-halophytic. Organisms that thrive in hypersaline environments possess specific mechanisms to adjust their internal osmotic status. One such mechanism is the ability to accumulate low molecular weight organic compatible solutes such as sugars, some amino acids and quaternary ammonium compounds. Other mechanisms of adaptation to high salinity is the exclusion of Na+ ion from the sodium sensitive sites which has been proposed as a function of an Na^+/H^+ antiporter and Na^+ ATPase. The results of expression of compatible solute, glycine betaine, and heterologous sodium efflux transporter, Na^+/H^+ antiporter, will be discussed in relation to salt tolerance of plants.

In addition to these toxic effects, salt stress also causes the induction of oxidative stress. Upon salt stress, stomatal closure triggered by abscisic acid limits CO_2 supply to the leaf leading to overreduction of the photosynthetic electron transport (ET) chain. The overreduction of ET chain causes the generation of active oxygen species such as singlet oxygen, superoxide anion, hydrogen peroxide, and hydroxyl radical. Therefore, the enhancement of enzyme activity involved in active oxygen scavenging systems may be a potent strategy to increase salt tolerance. The results of transgenic plants overexpressing SOD and glutamine synthetase will be presented. Finally, the role of the heat shock protein DnaK/Hsp70 in salt tolerance will be discussed.

Salt tolerance of a freshwater *Synechococcus* sp. PCC 7942 transformed with a shuttle vector, that contains the 1.4 kb fragment encoding a *Vibrio alginolyticus* Na^+/H^+ antiporter, was investigated. Northern blot and reverse transcript-PCR analysis showed the expression of the *Vibrio nhaAv* gene in *Synechococcus*.

Increased activity of the Na^+/H^+ antiporter in the transformant was observed by using acridine orange fluorescence. In growth medium containing low concentrations of NaCl, the growth rate of the transformant was similar to that of the control cells, but it became lower under high concentrations of NaCl, indicating the sodium sensitivity of the transformant. Electron transport activities and the levels of ATP and Chl were lower in the transformant at high salinity. The intracellular Na+ level was 2.4 times higher in the transformant. Acridine orange fluorescence analysis of thylakoid membranes in *Synechococcus* cells revealed the functional expression of the *Vibrio* Na^+/H^+ antiporter in thylakoid membranes. *E. coli* cells transformed with *Vibrio nhaAv* exhibited sodium tolerance. The expression of the *nhaAv* gene conferred tolerance to LiCl although not to NaCl in *Synechococcus* cells.

The potential role of SOD in the protection against salt stress was examined using transgenic rice plants. The coding region of the yeast mitochondrial Mn-SOD gene was fused with the chloroplast targetting signal of glutamine synthetase gene and introduced into rice protoplasts by electroporation. Immunogold labeling experiments revealed that the yeast Mn-SOD was accumulated in the chloroplasts of transgenic rice. Total SOD activity in the control plant was mostly attributed to the activity of cytosolic and chloroplastic Cu/Zn-SOD. Total SOD activity in the transformant was about 1.7-fold that of the control under non-stressed conditions. The photosynthetic electron transport rates of control and transgenic rice were similar under non-stressed conditions. Upon salt stress (100 mM NaCl), the SOD activities decreased in both plants, but decreased faster in the control plant. The activities of overexpressed Mn-SOD and cytosolic Cu/Zn-SOD did not change upon salt stress in either the transgenic or control plants, whereas the chloroplastic Cu/Zn-SOD activity in control rice decreased significantly. At high salinity, the ascorbate peroxidase activity of the transformant was about 1.5-fold higher than that in the control. These results suggest that increased levels of ascorbate peroxidase and high levels of chloroplastic SOD in the transformant are important factors for salt resistance in rice.

Previously, it was found that the *dnaK1* gene of a halotolerant cyanobacterium *Aphanothece halophytica* (*A. halophytica*) encodes a polypeptide of 721 amino acids which has a long C-terminal region rich in acidic amino acid residues. To understand whether the *A. halophytica* DnaK1 possesses chaperone activity at high

salinity and to clarify the role of the extra C-terminal amino acids, a comparative study of three kinds of DnaK molecules for the ATPase activity as well as the refolding activity of other urea-denatured proteins were examined under various salinity conditions. DnaK1s from *A. halophytica* and *Synechococcus* sp. PCC 7942 and the C-terminal deleted *A. halophytica* DnaK1 were expressed in E. coli and purified. It was found that the ATPase activity of *A. halophytica* DnaK1 was very high even at high salinity (1.0 M NaCl or KCl), whereas this activity in *Synechococcus* PCC 7942 DnaK1 decreased under increasing concentrations of NaCl or KCl. The salt dependence on the refolding activity of urea denatured lactate dehydrogenase by DnaK1s was similar to that of ATPase activity of the respective DnaK1s. The deletion of the C-terminal amino acids of *A. halophytica* DnaK1 had no effect on the ATPase activity, but caused a significant decrease in the refolding activity of other denatured protein. These facts indicate that the extra C-terminal region of *A. halophytica* DnaK1 plays an important role in the refolding of other urea-denatured proteins at high salinity. Furthermore, it was shown that DnaK1 could assist the copper binding of precursor apo-plastocyanin as well as that of mature apo-plastocyanin during the folding of these copper proteins.

To test the role of the heat shock protein DnaK/Hsp70 in salt tolerance, we made transgenic plants of *Nicotiana tabacum* cv. Petit Havana SR1 with DnaK1 from a halotolerant *cyanobacterium Aphanothece halophytica* overexpressed in the cytosol. The growth rate and photosynthetic activities of the transgenic and control tobacco plants were similar under non-stressed conditions. The CO_2 assimilation rate of the control plants decreased with increasing concentration of NaCl. After 3 days of treatment with 0.6 M NaCl, the CO_2 fixation rate decreased to 40% of that in the non-stressed plants whereas its activity in the transgenic plants was about 85% of that in the non-stressed plants. Similar results were observed for the stomatal transpiration. The sodium contents in leaves of the control plants were significantly increased by salt stress whereas those in the transgenic plants remained at levels similar to those in the non-stressed plants. Total protein contents and RuBisCO levels were decreased by salt stress in both the transgenic and control plants but the decrease was slight in the transgenic tobacco. All these data clearly indicate that the expression of DnaK1 from a halotolerant cyanobacterium *Aphanothece halophytica* improved the salt tolerance of the tobacco plant.

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