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EXPRESSION OF MANGROVE TCP-1 IN E. COLI AND YEAST

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Functional screening of cDNAs encoding proteins essential for the salt-tolerance in mangrove plant, *Bruguiera sexangula*, was performed using *Escherichia coli* as a host organism. A cDNA encoding TCP-1 homolog was isolated in this screening. This cDNA showed 79.6% similarity to TCP-1 cDNA of *Arabidopsis thariana*. In general, TCP-1 is known to be one of a subunit of CCT (chaperonin containing TCP-1), and play a critical role on the ATP-dependent polymerization of actin and tubulin in eucaryotic cell. On the other hand, there are no informations about the function of TCP-1 for the salt-resistance. In this study, the new function of TCP-1 was investigated. Site directed mutagenesis analysis of mangrove TCP-1 indicated that ATP binding region is not required for the salt-tolerance function. Wheather TCP-1 from other plants have salt-tolerance function in *E. coli* or not, *A. thaliana* TCP-1 cDNA was cloned by PCR and the activity was examined. *A. thaliana* TCP-1 also showed the activity to enhance the salt-tolerance in *E. coli*. From these results, plant TCP-1 may have a function for enhancement of the salt-tolerance in *E. coli*.

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Gene expression profiling of salinity stress responses using expressed sequence tag (EST)-based microarrays in the common ice plant, *Mesembryanthemum crystallinum*.

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Mesembryanthemum crystallinum, a facultative CAM halophyte, can shift from C_3 to Crassulacean acid metabolism (CAM) photosynthesis following exposure to environmental stresses such as high salinity and water deficit. We have simultaneously analyzed changes in the expression pattern of ~3000 selected genes in response to salt stress (500 mM NaCl) using EST-based microarrays. Approximately 30% of transcripts showed significant up- or down-regulation from 3 to 72 hours following the imposition of salinity stress. The most pronounced changes in transcript abundance were observed at 48 hours after stress, a time that corresponds temporally to the buildup of CAM enzymatic machinery. Several hundred unique transcripts showed at least a two-fold increase in abundance, whereas in excess of 200 genes were down regulated to a similar extent. Up-regulated genes were mainly involved in photosynthetic processes. Surprisingly, more than 30% of the induced transcripts were functionally unknown, including many novel genes. The detailed temporal dynamics of salinity stress responses revealed by this study provide novel insights into the perception and response mechanisms that contribute to survival following exposure to high salinity stress.

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PURIFICATION AND CHARACTERIZATION OF β − AMYLASES INDUCED BY WATER STRESS IN CUCUMBER COTYLEDONS

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Water stress is one of the most serious problems for immotile plants and, in order to survive, plants show numerous metabolic and molecular responses to water stress. We previously reported that β-amylase activities were enhanced by water stress in cucumber cotyledons[1]. The present study has been undertaken to characterize the β-amylases biochemically.

Amylolytic enzymes were partially purified from the extracts of air-dried (6h) or water-treated (6h, as a control) cucumber cotyledons by DEAE-Toyopearl column chromatography, glycogen precipitation and gel filtration on Superdex 200 pg. Two distinct peaks of amylolytic activities (105 kD [A-I], 40 kD [A-II]) were separated by gel filtration on Superdex 200 pg. The amylolytic enzymes in both A-I and A-II were β-amylases, not α-amylases because they could degrade PNPG5 but not BPNPG7. Some β-amylase spots were detected when A-II was analyzed by activity staining after two-dimensional (IEF and Native) PAGE. In particular, four β-amyalse isozymes (pI 5.8 ~ 6.8) among them were strongly induced by drought stress. It was suggested that these four β-amylases may have an important role in the response to water stress.

1) Todaka et al. (2000) J. Experimental Botany 51: 739-745

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Functional analysis of galactinol synthase in *Arabidopsis* in drought stress tolerance

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Raffinose Family Oligosaccharides (RFO) is thought to function as an osmoprotectant in developing seeds. However, little is known about the function of RFO in plants. In this study, we attempted to elucidate the function of RFO in plants under water stress conditions.

Sugar analysis showed that drought-, high salinity- and cold-treated *Arabidopsis* plants contain a large amount of raffinose, which is barely detectable in non-stressed plant. This suggests that raffinose functions as an osmoprotectant in plants as well as in developing seeds.

Galactinol synthase catalyzes the first committed step in the biosynthesis of RFO. We isolated three stress-responsive galactinol synthase genes from *Arabidopsis* and named them *AtGolS1, 2 and 3*. The *AtGolS1, 2 and 3* genes were expressed in *Escherichia coli* to obtain their GST fusion proteins. The GST-AtGolS1,2,3 fusion proteins had galactinol synthase activities. We also analyzed transgenic *Arabidopsis* plants for *AtGolS1*, and *AtGolS2*. The *AtGolS2* overexpressors accumulated galactinol and raffinose, and aquired drought tolerance. By contrast, antisense suppression of *AtGolS1* reduced galactinol and raffinose contents and showed drought-sensitive phenotype.