F255

Identification of Genes Involved in K^+ Nutrition using Comparative Proteome Analysis

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The efficient uptake and utilization of inorganic nutrientsfrom soil are critical for plant's survival and crop productivity. K+ is the most abundant cation and one of three major nutrients in plants. To understand the K^+ nutrition in plants at the molecular level, we have screened the differentially expressed proteins in seedlings treated with 10 M (deficient condition) and 2 mM K+(normal condition) in growth medium for 3 h (early induction phase) and 3 d (steady state phase) using proteome analysis. In proteomics approach using panoramic gels composed of four-1 pH unit gels ranging pH 4-7, we identified 108 protein spots differentially expressed by K+deficiency after 3 h and 3d. Among those, there are putative transcription factors, proteins possibly involved in protein degradation, and putative G-proteins possibly involved in signal transduction. To understand the role of these proteins *in planta*, we are constructing the systematic transgenic plants harboring genes of our interests. Also to study in vivo functions of genes, we obtained knockout mutants carrying each genes disrupted by T-DNA insertion.[Supported by a grant (code CG1323) from Crop Functional Genomics Center of 21st Century Frontier Research Program]

F256

Molecular Chaperone Activity of a Tobacco Small Heat Shock Protein, NtHSP18.3.

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Plant synthesizes variable small heat shock proteins (sHSPs) that have been grouped into more than five classes. Among sHSPs, cytosolic class I sHSPs have been most extensively studied for the functional mode. However, the mechanism needs to be further elaborated. We isolated a genomic clone, NtHSP18.3, for a cytosolic class I sHSP from *Nicotiana tabacum* and the open reading frame was expressed in *Escherichia coli*. The protein, NtHSP18.3, was purified from *E. coli*, and the functional mode was examined. NtHSP18.3 prevented aggregation of malate dehydrogenase (MDH) and citrate synthase (CS) at high temperature in *in vitro*. Interaction between NtHSP18.3 and the target proteins in this condition was such that the denatured substrates coated the NtHSP18.3 oligomer to form expanded complexes. When the range of target proteins was expanded to total *E. coli* soluble proteins, much of the *E. coli* proteins was protected from heat-aggregation by NtHSP18.3. These results suggested that NtHSP18.3 is a molecular chaperone with a broad substrate range.

F257

Isolation of High Temperature Stress-related Genes of Hot Pepper Plants

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High temperatures limit vegetal growth resulting in low plant yields. Plants respond to high temperature stress by synthesizing a set of proteins, which may be responsible for the acquisition of thermo-tolerance. In this study, hot pepper is being used as a model plant. To isolate genes, related to high temperature stress responses, hot pepper plants were grown in a growth chamber for 25 days and submitted to high temperature stress e.g 30 min, 1h, 2h, 3h, or 4h at 42℃. Poly (A) RNA was extracted, and a cDNA library was built. Among the cDNA clones isolated from a differential screening of the library to enrich high temperature stress-inducible genes, we report here four cDNA clones. These cDNA clones displayed either a significant nucleic acid or amino acid sequence similarity to sequences known in the GenBank. RNA blot hybridizations demonstrated that the expression of one dehydrin homolog, one protein disulfide isomerase, one putative ring finger zinc protein and one gene of unknown function are up regulated, i.e. high temperature stress-induced cDNA clones. Results in this study contribute toward the on-going attempt to isolate high temperature stress-induced novel genes in hot pepper.

F258

The Effects of Polyamine and Environmental Stresses on Cell Cycling in BY-2 Tobacco Cells

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The polyamines putrescine, spermidine and spermine are positively charged, low molecular weight compounds that occur in both prokaryotic and eukaryotic cells. We have chosen tobacco as the experimental system because of the high degree of synchronization attainable in the tobacco Bright Yellow-2 (BY-2) cell line. There weredetermined the effects of exogenous polyamines on the division in synchronized BY-2 cell cultures. The cell cycle profile of cells treated with 1mM putrescine, spermidine, and spermine was essentially unchanged compared with control cells until 24h. But, DNA content of S phase before reaching the G2 phase constantly increase after 24h of treatment polyamines, suggesting polyamines increased the continuity of cell cycling. Also, polyamine induced more amounts of transcripts for cyclins. Other plant growth regulators, kinetin, auxin, brassinosteroid, abscisic acid, jasmonic acid, and gibberellin, were differently changed the profile of cell cycling in BY-2 cells. Cell cycle arrest was associated with inhibition of the activity of cyclin-dependent kinase and of gene expression for cyclins after treatment with a various stresses such as salt, cold, heat, etc. Possibly, this dual response to osmotic and oxidative stress could mirror an evolutionarily conserved response to environmental stresses and thereby provide a good model to study the molecular events induced by specific environmental stresses that influence cell division.