F211

Functional Characterization of *CaHK1*; a Putative Pepper Osmosensor

Hyung-Sae Kim[P], Jae Joon Kim[1], Jee Hyun Lee[1], Sung-Soo Jun[1], Young-Nam Hong[C]

School of Biological Sciences, Seoul National University, Seoul 151-742

A partial cDNA clone homologous to Arabidopsis osmosensor (*AtHK1*) was isolated from cDNA library of hot pepper. Subsequently, a full-length cDNA was isolated by 5'-RACE. The complete clone was coding for histidine kinase, and thus was named as *CaHK1* (*Capsium annum* Histidine Kinase 1). *CaHK1*was composed of 4035 bp with an open reading frame of 1215 aa. The protein contains a phosphoacceptor domain, two transmembranal domains, a histidine kinase domain and response regulator receiver domain. *CaHK1* was constitutively expressed, but in a higher level in roots. Upon PEG-treatment, *CaHK1*was down-regulated in the roots within 1 h while in the leaves it was initially up-regulated and subsequently down-regulated. After NaCl-, ABA-, or cold-treatment *CaHK1* expression was substantially increased in the beginning and then was steadily decreased to the initial level and a negligible level after 24 h in leaves and roots, respectively. Using genome walker PCR, a 2 Kb-long DNA fragment comprising upstream of *CaHK1*ORF in the promoter region was isolated containing 11 stress responsive elements. To understand the role of osmosensor (*CaHK1*) in plants, transgenic *Arabidopsis* suppressing *AtHK1* by post-transcriptional gene silencing and transgenic *Arabidopsis* overexpressing full length *CaHK1*, or partial *CaHK1*each only containing acceptor, kinase or receiver domain are generated. The initial data indicated that *AtHK1*suppressed Arabidopsis *were more vulunerable to dehydration.*

F212

Improved Tolerance and Photosynthetic Capacity under Abiotic Stresses in Transgenic Tobacco Overexpressing *Ca*LEA Protein

Jee Hyun Lee[P], Jae Joon Kim[1], Hyung Sae Kim[1], Anna Joe[1], Sung-Soo Jun[1], Young-Nam Hong[C]

School of Biological Sciences, Seoul National University, Seoul 151-742

To understand the role of *Ca*LEA protein, the product of a dehydration-responsive gene from hot pepper, *CaLEA*-overexpressing transgenic tobacco plants were generated and their physiological and photosynthetic responses under various abiotic stresses were examined. *CaLEA*-overexpressing tobacco plants exhibited enhanced tolerance against dehydration, administered by limited water supply or PEG-treatment. In addition, leaf O2evolution was less inhibited in *CaLEA*-producing plants. Transgenic plants also showed improved tolerance against high temperature stress. *CaLEA*-producing plants maintained better photosynthetic activities in leaf O2evolution, the photosynthetic efficiency of PSII (Fv/Fm), and photochemical activity (qP). *CaLEA*-producing transgenic plants also survived better under salt stress. Loss in Chl content after salt-treatment was much delayed in *CaLEA*-producing plants and the photosynthetic activities were maintained better. In conclusion, *Ca*LEA protein appears to be a wide-ranged stress protectant in plants against not only water stress but also high temperature and salt stress.

F213

Effects of Spermidine on Antioxidant Defense System in the Chloroplast of Paraquat-treated Radish

Sun-mi Ahn[P], Chang-Duck Jin[C]

[P]Department of Biological Education, Kangwon National University, Chunchon 200-701; [C]Division of Biological Sciences, Kangwon National University, Chunchon 200-701

The relationship between a protective role of exogenous spermidine(Spd) and some antioxdant systems were investigated in the chloroplast of Paraquat(PQ)-treated radish (*Raphanus sativus* L. cv. Taewang) cotyledons. PQ alone treatment caused significant oxidative damages involving the losses of chlorophyll and stroma and thylakoid proteins as well as induction of lipid peroxidation and hydrogen peroxide accumulation. Also, PQ resulted in the rapid decrease of ascorbate content with an apparent increase of dehydro-ascorbate, and caused an weak loss of glutathione level. However, pretreatment with Spd prevented all of these changes. PQ treatment led to a strong decrease of ascorbate peroxidase(APX), while dehydroascorbate reductase(DHAR) and glutathione reductase(GR) were significantly induced. However, Spd pretreatment inhibited a rapid decrease of APX and reduced DHAR and GR responses to PQ. The possible role of Spd and antioxidant systems in response to PQ stress in the chloroplast was discussed.

F214

Mass Analysis of Oxidative Stress-related Genes Using EST and Microarray in *Capsicum annuum* L.

Nam Houn Lee[P], Sang Ho Lee[1], Hyoungseok Lee[1], Cheol-Goo Hur[2], Tae-Hoon Chung[2], Sunyong Park[2], Chung Sun An[C]

[PC1]School of Biological Sciences, Seoul National University, Seoul 151-742; [2]Korea Research Institute of Bioscience and Biotechnology, Daejeon 305-333

For the massive screening of the genes induced by oxidative stress, we constructed a cDNA library from hot pepper (*Capsicum annuum*L. cv. Nockkwang) leaves treated with paraquat. From this cDNA library, 1,589 cDNA clones were sequenced from their 5' ends. The sequences were clustered into 1,252 non-redundant groups comprised of 152 non-redundant clusters and 1,100 singletons. Similarity search results using NCBI protein database identified 1005 ESTs (80.27%) as known proteins, 197 ESTs (15.73%) as unknown and 50 ESTs (3.99%) as no hit. In the ESTs, oxidative stress-related genes such as ascorbate peroxidase, catalase, osmotin precursor were highly expressed. Functional grouping using MIPS database revealed that the majority of the ESTs are belongs to several categories such as metabolism, protein synthesis, cellular communication/signal transduction, cell rescue and defense. The microarray using 1265 cDNA clones were used to study the transcriptome related to oxidative stress. Analyses of the hybridization revealed that 28, 39 and 62 genes were up-regulated and 11, 40 and 21 genes down-regulated in 1, 6 and 12 h, respectively. 145 representative cDNAs were subject to hierarchical and k-means clustering to analyze gene expression profiles and clustered into 12 groups. 12 clustered groups showed different expression patterns. The expression pattern for the several cDNA clones was analyzed by RNA gel-blot method to check the reproducibility of cDNA microarray data.