E243 Cloning, Expression and Genomic Characterization of Three Catalase Genes from Small Radish (*Raphanus sativus* L.)

Soon Il Kwon*, and Chung Sun An
Department of Biology, Seoul National University

We have isolated three catalase genes (cat1, cat2, and cat3) from cold-treated seedling cDNA library of small radish through PCR method. Their nucleotide and amino acid sequences showed the highest homology those of *Arabidopsis*. Small radish catalase could be resolved into two major isozyme forms in different tissues by nondenaturing polyacrylamide gel staining. Southern-blot analysis of genomic DNA showed that cat1 and cat2 are members of small multi-gene families. Northern analysis of catalase genes expression was performed on RNAs extracted from different tissues. The expression of catalase genes exhibited circadian rhythm upon illumination but not in etiolated seedlings, and influenced by various chemicals (paraquat, plumbagin, cercosporin, sucrose, mannitol, salt..), hormones (ABA, IAA) and light sources (white light, U.V.). Cat1 and Cat2 genomic clones have been isolated and their complete DNA sequences were determined. In order to determine tissue-specific expression pattern of the cat1 gene, promoter-reporter gene fusion constructs were made by fusing 2.0 kb of the cat1 5′ UTR sequence to the coding region of the GUS gene. These fusion construct was introduced into *Arabidopsis thaliana* cv. columbia and the expression of this construct in various tissues is being examined.

E244 Characterization and Functional Analysis of Antifungal Protein cDNA Clone from Hot Pepper

Yeon Mi Lee*, and Chung Sun An
Department of Biology, Seoul National University

We obtained an antifungal protein cDNA clone by cDNA library screening from *Capsicum annuum* L. The clone, termed pCaAFP, encodes for 85 amino acids including 8 cysteine residues as a full insert size of 509 bp. The putative protein was consisted of three domains : signal peptide, chitin binding domain, and C-terminal peptide. Southern blot analysis of genomic DNA showed that AFP gene existed as a single copy, but some other related signals showed up suggesting the presence of other copies. Northern blot analysis revealed that CaAFP mRNA was expressed in the tissue-specific and developmentally regulated manner. For its functional analysis, AFP gene was cloned into an *E. coli* expression vector, pET-30b(+), under the control of T7 promoter. Expressed fusion protein was purified by using His · tag affinity column chromatography and tested on several fungal species. Purified AFP fusion protein inhibited the germination and appressorium formation of several plant pathogenic fungi. Localization and the functional analysis of the protein are being undertaken to elucidate its function in hot pepper.