F210 Molecular Cloning and Characterization of a Putative Lipid Transfer Protein from Hot Pepper.

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To better understand the anther development from the flowers of *Capsicum annuum*, several anther-specific cDNAs were isolated by DDRT-PCR (differential display reverse transcriptase- polymerase chain reaction). Among those clones, a cDNA of 0.6 kb was studied in detail. Nucleotide sequence analysis of the clone revealed that the clone contains an open reading frame of 123 amino acids which exhibits 23 ~ 60% identity with those reported for the non-specific lipid transfer protein (nsLTP) genes from other species. Based on the sequence homology and the presence of 8 conserved Cys residues found in other LTPs, the clone was named as *CaLTP*. Northern blot analysis of the clone confirmed that *CaLTP* mRNA expression is preferential or specific to anther tissue. The hydropathy profile analysis shows that the N-terminal 21 amino acids of the CaLTP protein has the characteristics of a signal peptide for protein secretion or targeting into dense microbody-like vesicles. Southern hybridization analysis reveals that *CaLTP* is present as a single-copy or a member of small multigene family in hot pepper. To elucidate the *in vivo* function(s) of CaLTP, the protein is currently expressed in *E. coli* as a fusion protein. Anther-specificity of *CaLTP* related to the function of the protein will be discussed.

F211 Characterization of Genomic Clones of Anthocyanidin Synthase from Apple

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Anthocyanidins, the first colored compound in anthocyanin biosynthetic pathway, are converted from leucoanthocyanidins by anthocyanidin synthase (ANS, leucoanthocynidin dioxygenease) enzyme. The reaction is thought to include dehydration and an oxidation step. In order to understand the anthocyanidin biosynthesis mechanism in fruits, a cDNA (*MdANS*) encoding ANS was cloned and characterized from apple (*Malus domestica* Borkh. cv. Fuji). It has been shown that *MdANS* was preferentially expressed in the skin tissues of fruits and greatly induced on the tissues by light exposure. Using the 1.3 kb *MdANS* cDNA as a probe we screened ca. 3.0×10^5 recombinant plaques of an apple genomic library. Nine positive clones after tertiary screening were divided into four groups (λ ANS1 ~ λ ANS4) based on the restriction mapping and partial nucleotide sequencing. An *Eco*RI fragment (λ ANS1.5) of 1.5 kb from λ ANS1 contained the exactly matching 0.1 kb of 5′ UTR of *MdANS* indicating that λ ANS1 is the genomic version of *MdANS*. Another *Eco*RI fragment (λ ANS2.0) of 2.0 kb from λ ANS2 contains a region which is 86% homologous to the 5′ UTR of *MdANS* indicating that λ ANS2 is a member of ANS multigene family. It is expected that the remaining two genomic clones are other members of the ANS gene family since Southern analysis has shown that *ANS* are composed of 3~4 members of gene family in apple. λ ANS1.5 and λ ANS2.0 containing the promoter region is currently ligated into β -glucuronidase (GUS) reporter gene and transformed into *Arabidopsis* and tobacco plants. Transient expression pattern of the ANS promoter-GUS construct using helium bombardment in fruit tissues will be presented.