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Molecular Cloning and Characterization of Anther-expressed Cysteine Protease Gene, *rCysP1*, from T-DNA Tagging Rice

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Cysteine proteases play essential roles in a variety of developmental and physiological processes in many plants. In rice (*Oryza sativa*), oryzain is a primary cysteine protease responsible for the digestion of seed storage proteins to provide nutrients to support the growth of young seedlings. In the present study, we isolated and characterized an anther-expressed gene from a pool of T-DNA tagging rice lines. Sequence analysis revealed that the gene designated, *rCysP1*, was 1,473 bp long with an open reading frame of 490 amino acid residues that show a significant homology to cysteine proteases. Southern hybridization analysis indicated that the *rCysP1* was present as a single copy in the rice genome. Molecular characterizations and function of the gene are shown and discussed, respectively.

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Study of Pistil-expressed Genes from Brassica Campestris

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Chinese cabbage is one of the major vegetable crops in Korea. Our primary goal in this study is to identify and utilize useful genes that are expressed in the pistil of Chinese cabbage. In order to do this, we have constructed a pistil cDNA library of Chinese cabbage and used the library to collect cDNA ESTs. A total of 864 cDNA EST was collected from a pistil cDNA library of Chinese cabbage. After elimination of 20% redundant ESTs from the total, 690 cDNA ESTs were used in profiling. Among the 13 profiling categories, the results from Blastx search revealed that more than 50% of the pistil genes were functionally not clear. It was also found that the genes in organ (pistil) specific, signal transduction, regulatory protein, and metabolism were 7%, 4.8%, 6.38%, and 18.7%, respectively. Currently, we are under study of few pistil genes that are economically useful once they are utilized.

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Molecular Genetic and Biochemical Analyses of Loss-of-Function Mutants for *AtCYP51* Encoding Obtusifoliol 14-Demethylase in Sterol Biosynthesis

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Plant *CYP51* encodes obtusifoliol 14-demethylase involved in the early step of sterol biosynthetic pathway. Arabidopsis genome contains two *CYP51A* genes, *CYP51A1* and *CYP51A2*with 72% of amino acid sequence identity. RT-PCR and GUS histochemical analyses showed a differential gene expression pattern between the two genes. As the first step to study the molecular genetics and biochemical characteristics of both *CYP51A* genes, we screened Arabidopsis seed pools generated from T-DNA insertional mutagenesis using systematic reverse genetics. From the screening of about 120,000 T-DNA insertion lines, we isolated 1 mutant allele (*cyp51A1*-1) for *CYP51A1* and 3 mutant alleles (*cyp51A2*-1 to 3) for *CYP51A2*, respectively. *cyp51A2* mutant showed multiple defects in plant growth and development such as stunted hypocotyl and root, reduced cell elongation, seedling lethality (or reduced growth rate in the weak allele) and sterility. The phytochemical analysis for *cyp51A2*-3 mutant revealed that the mutant accumulates the direct substrate, obtusifoliol, and further upstream metabolitessuch as cycloartenol and 24-methylene cycloartenol. In contrast, the phytosterol contents were greatly reduced in the mutant.

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Characterization of T-DNA insertional Mutant of ABA-inducible Phosphatase Gene in Rice

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T-DNA insertional mutant lines tagged by the gene trap vector pGA2707 have been investigated by the end sequencing of T-DNA. One of the mutant line in which T-DNA inserted in the phosphatase gene was chosen to study the phenotype alterations. This phosphatase is highly homologous with the ABA-inducible phosphatase in beechnut seed. Thus, the transcripts level of this phosphatase gene in the presence of ABA has been checked.The position of T-DNA insertion is determined by the iPCR and it is revealed that the T-DNA inserted in the second intron of the phophatase gene. This phosphatase is type 2C which requires Mg^{+2} as a cofactor. There are at least 3 copies of this phosphatase gene in the rice genome, but their substrates may be different. The morphological phenotype of the mutants is quite different with the wild type. Their height is just half of the wild type, but their roots are quite long comparing to the roots of wild type. These mutant lines are all dead around four weeks after germination. NCBI Blast search revealed that this phosphatase has a very high similarity with the ABA-inducible phosphatase in beechnut dormant seeds. Thus the RT-PCR analysis of the transcripts level of this phosphatase in the presence of ABA is on progress.