P 44 Identification and Characterization of Flavanone 3-Hydroxylase (F3H) Gene from *Chrysanthemum morifolium* (syn. *Dendranthema gradiflora*)

KIM, Young-Hee*

Dept. of Bioinformatics, Sangmyung University, Cheonan 330-180, Korea

Objectives

A cDNA clone for a flavanone 3-hydroxylase (F3H) gene was isolated from *Chrysanthemum morifolium*. The cloned F3H gene is 1,262 bp long encoding 357 amino acids. The deduced nucleic acid sequence shows 73% identity to F3H gene of *Arabidopsis thaliana*. The reverse transcriptase polymerase chain reaction (RT-PCR) shows that the F3H gene is expressed in yellow petal of *Chrysanthemum morifolium* while the gene is not expressed in white petal of *Chrysnathemum morifolium*. Low stringency of DNA blot analysis indicated that there is a single copy of gene encoding F3H in *Chrysanthemum morifolium*.

Materials and Methods

Total RNA was isolated from petals of *Chrysanthemun morifolium* using the method of Turpen and Griffith (1986). cDNA library was constructed using the λZAPII EcoR1/CIP-treated vector Kit (Stratagene # 236211) and screened. Putative plaques were further screened with the probes described above and were subsequently purified by plating recombinant phage at a lower density. Bluescript SK- plasmids containing the cDNA insert were obtained by *in vivo* excision as recommended by the manufacturer. Selected clones were digested with *Eco* RI, and then separated on a 1% agarose gel. Sequencing was performed in both strands using M13 forward and M13 reverse primers by auto-sequencing system (Pharmacia). After digestion with the appropriate restriction endonucleases, the DNA was subjected to electrophoresis through a 1.0% agarose gel and transferred to Hybond-N (Amersham) nylon membrane by capillary blotting according to the manufacturer's protocol. Hybridization and washing were done in SSC salt solution according to the manufacturer's protocol (Amersham). One microgram of mRNA from *Chrysanthemum morifolium* was reverse transcribed at 37℃ for 30 min by 100units of the M-MLV reverse transcriptase (Promega) in a 20 μL reaction mixture containing 100 pmole primer2, the corresponding buffer (Promega), 0.25 mM dNTP, and 10 units of RNasin (Promega). PCR was performed during 35 cycles of 1min denaturation at 94℃, 1min annealing at 55℃, and 1min polymerization at 72℃.

Results and Discussion

Three clones were found from first screening. Two different insert sizes were found, and they were about 1.2 Kb and 1.3Kb length. The sequence of the 1.3 kb clone was determined by automatic sequencer (pharmacia). The length of insert was 1,262 bp and 357 amino acids was coded in the ORF region. The start codon is in the 40 bp and stop codon is in the 1110 bp from 5′ -end of cDNA clone. There is no poly A tail in the 3′ -end, but the canonical polyadenylation sequence, AATAAA, exists in the 1179 bp from 5′ -end of the clone. It shows high identity in the level of nucleic acid sequence, when compared with sequences of known F3H cDNA clones. Yellow colored petal showed a prominent band with 1.3 Kb size, while white colored petal did not. This result seems that F3H is more expressed in yellow colored petal than white colored one. And, it may be possible to control the flower color by modifying activities of enzymes involved in flavonoid biosynthetic pathway. In future study, we will examine how F3H is regulated according to the condition change by white light and also observe the phenotypic variation between F3H sense- and anti-sense transformants.

*Corresponding author. Tel 041-550-5287 E-mail ykim@smuc.ac.kr