

# MOLECULAR CLONING AND CHARACTERIZATION OF ANTHOCYANIN BIOSYNTHESIS GENES IN THE FUJI APPLE (*Malus domestica* cv. Fuji)

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Anthocyanins, a subclass of flavonoids, have been implicated as major color pigments in flowers and fruits. We have isolated and characterized several genes involved in anthocyanin biosynthesis from apple (*Malus domestica* cv. Fuji). The cDNA clones encoding flavanone 3-hydroxylase (F3H) and anthocyanidin synthase (AS) were screened from the Fuji apple peel cDNA library. A probe for F3H was generated from the peel cDNA using known sequences. The AS probe was obtained from the peel cDNA using degenerate oligonucleotides as PCR primers. The dihydroflavonol 4-reductase (DFR) cDNA was isolated by random sequencing of young fruit cDNA clones. In order to understand the relationship between anthocyanin accumulation and the gene expression, we have performed RNA blot analysis. Total RNA was extracted from peels of six different stages of fruit development. The expression of DFR was gradually increased from 0 DAD (day after decapping of paper bags) until 11 DAD and the level decreased thereafter. In contrast, the AS expression was rarely detected at 0 DAD, reached maximum at 1 DAD and maintained until 11 DAD. The expression was decreased dramatically to a minimal level at 20 DAD. However the anthocyanin level gradually increased until 20 DAD when the fruit was harvested. The expression of the genes was investigated using different tissues. Compared to DFR, AS expression was preferential to peel tissues and minimally detected in seedlings, leaves and hypanthia. These results indicate that the AS and DFR expression in apple are differentially regulated. The study on other clones is progressing.