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TOWARD METABOLIC ENGINEERING OF APIGENIN PATHWAY IN *MATRICARIA RECUTITA*

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Chamomile (*Matricaria recutita*), a member of the *Asteraceae* family, is a good source of health-related compounds such as sesquiterpenes, coumarins, polyacetylenes and flavonoids including apigenin. This flavone exhibits interesting *in vitro* and *in vivo* pharmacological activities as anti-oxidant, anti-inflammatory, and anti-cancer properties. Moreover, recently it has been established that apigenin significantly decreased the blood levels of total and low-density lipoprotein cholesterol in mice. Apigenin is synthesized *in planta* by the phenylpropanoid pathway only in very limited amounts.

In this study we investigated the apigenin pathway in chamomile in order to pave the way to increase the production of this valuable compound by metabolic engineering approches. For this purpose, the full-length cDNA of flavone synthase gene (*FNS*), encoding the key enzyme of the apigenin pathway, was isolated from *M. recutita* using a similarity-based cloning strategy. Nested PCRs with degenerate primer sets, designed to match the highly conserved regions of other *FNS* genes, were carried out to amplify the core fragment of 510 bp. PCR-RACE was applied for amplification of 3' and 5'-ends. Comparative analysis showed that chamomile *FNS* sequence was closely related to other flavone synthases, showing a high degree of amino acid identity with flavone synthase II of *Cynara cardunculus* var. *scolymus.* Expression pattern of the *M. recutita* flavone synthase gene was achieved by Real-Time in roots, stems, leaves and flowers.

In addition, a method of stable genetic transformation by *Agrobacterium tumefaciens* was set up in *M. recutita* using the LBA4404 strain harboring a binary vector with marker gene neomycin phosphotransferase (*NPTII*) and reporter gene β-glucuronidase (*GUS*). This protocol could become a useful tool for metabolic engineering approches in chamomile.