

## ISOLATION, FUNCTIONAL CHARACTERIZATION AND MAPPING OF A *P*-COUMAROYL ESTER 3'-HYDROXYLASE GENE (C3'H) IN GLOBE ARTICHOKE

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*Cynara cardunculus* var. *scolymus*, C3'H, phenylpropanoid metabolism, genetic mapping

Globe artichoke (*Cynara cardunculus* L. var. *scolymus*) is a perennial and cross pollinated vegetable, native to the Mediterranean area, which is usually vegetatively propagated. The major use of globe artichoke is for human food, but various studies have demonstrated health-promoting properties of its leaf extracts, mainly due to their polyphenolic content. The dominating phenolics are di-caffeoylquinic acids and chlorogenic acid (5-caffeoylquinic acid).

The metabolism of dicaffeoylquinic acid in plant is still unknown, and the definition of chlorogenic acid synthesis remains controversial. Both chlorogenic and dicaffeoylquinic acids derive from the phenylpropanoid pathway and contain an -OH at 'meta' position on aromatic group. The CYP98 cytochrome P450 monooxygenase gene family is the most likely candidate class of enzymes catalyzing the 3'-hydroxylation of phenolic compounds.

By applying a CODEHOP strategy, we isolated and characterized the full-length cDNA of the globe artichoke C3'H (*p*-coumaroyl ester 3'-hydroxylase) gene, involved in both chlorogenic acid and lignin syntheses. Phylogenetic analyses demonstrated that this gene belongs to the CYP98 family.

Functional analysis was performed by expressing the C3'H gene in yeast with an *Arabidopsis thaliana* cytochrome P450 reductase *ATR*. The gene was found very active in converting the *p*-coumaroylshikimate into caffeoylshikimate. In contrast, the conversion of *p*-coumaroylquinic acid into caffeoylquinic acid was very slow and only detectable at a high concentration of substrate (100  $\mu$ M).

The C3'H promoter sequence was analyzed for identifying potential regulatory elements; a putative TATA box was found 33 bp upstream of the transcription start site and a putative CAAT box at 145 bp upstream. Real Time PCR analysis demonstrated higher expression level of C3'H following exposure to UVC, which we found to induce increases in dicaffeoylquinic acid biosynthesis.

We analyzed the allelic forms of C3'H gene in two globe artichoke genotypes: 'Romanesco C3' (a late maturing, non-spiny type) and 'Spinoso di Palermo' (an early maturing spiny type), which are the parents of an F<sub>1</sub> segregating populations we previously used for the development of the first genetic linkage map, based on a two way pseudo-testcross strategy. A SNP was identified making possible to localize the C3'H gene on the linkage group 10 of both maps, thus increasing the number of the shared intercross markers; The gene is located at about 2 and 7cM respectively from the microsatellite CELMS-39 and the AFLP markers P45/M47-06.