

ISOLATION OF A GERMACRENE-A SYNTHASE SEQUENCE FROM GLOBE ARTICHOKE

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The globe artichoke (*Cynara cardunculus* var. *scolymus* L.), a traditional component of the Mediterranean diet, contains bitter principles like sesquiterpene lactones (SLs); among them the most important are cynaropicrin and grosheimin, which explicate immuno-modulatory effects on the human immunitary system and prevent the invasion, migration and metastasis of leukocyte cancer cells.

Cynaropicrin and grosheimin belong to germacranolides, a complex family of molecules widespread in the *Asteraceae* (*Compositae*), which are thought to have originated from a common germacrene precursor: (+)-germacrene-A. To date, information concerning the gene responsible for the germacrene-A synthesis are available from chicory and *Artemisia annua*, however no information are available on genes involved in the additional steps (oxidation and cyclisation) required to convert germacrene-A to specific SLs (germacranolides, guaianolides and eudesmanolides).

We report on the isolation of germacrene-A synthase sequences in globe artichoke. Presumably, plant belonging to the *Asteraceae* family share common ancestor enzymes for the early steps of SL biosynthesis. Indeed, a comparative sequence analysis among the *Asteraceae* species showed similarity values up to 100% between available sequences from globe artichoke and the ones derived from yellow starthistle (*Centaurea solstitialis*) and safflower (*Carthamus tinctorius*). We retrieved germacreneA-Expressed-Sequence Tags from the EST database (<http://compgenomics.ucdavis.edu>) of starthistle and safflower (23k and 19k UNIGENES, respectively) by means of tBLASTn algorithm. Degenerate primers were designed on 6 conserved nucleotidic regions and were applied in PCR reactions using cDNA or gDNA of globe artichoke, as templates.

Three fragments of expected lengths were obtained by PCR amplification of foliar cDNA. They were cloned, sequenced and, when analysed by means of BlastX algorithm (non redundant protein database, Viridiplantae), they revealed very high identity values (from 84% to 93%) to the GAS gene described in chicory. Due to the presence of introns larger fragments were obtained using gDNA as a template.

The full length isolation of the globe artichoke GAS gene is currently in progress by applying 5'- and 3'-RACE; its enzymatic activity, as well as its expression in different plant tissues will be investigated.