BIOSYNTHESIS AND ACCUMULATION OF THE VALUABLE BIOACTIVE SESQUITERPENE LACTONE CYNAROPICRIN IN CYNARA CARDUNCULUS L.

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The plethora of bioactive compounds found in Asteraceae, such as terpenoids, alkaloids, phenolics and polyacetylenes make it a highly significant family both biochemically and pharmacologically. Sesquiterpene lactones (STLs) are one of the most prevalent and biologically significant class of secondary metabolite present, which show anti-carcinogen potential, anti-inflammatory capacity and provide plant defense against fungi, bacteria and insects.

In Cynara cardunculus, which includes the cultivated forms globe artichoke (var. scolymus) and cultivated cardoon (var. altillus), the principal constitutive STL is the cynaropicrin, which contributes to approximately 80% of the bitter taste.

In globe artichoke, we previously demonstrated that the Germacrene A synthase (CcGAS) catalyzes the first committed step in the costunolide biosynthesis. Here we report on the isolation and functional characterization of two Cytochrome P450 genes (CYP71BL5 and CYP71AV9) in a set of Cynara cardunculus unigenes. The encoded enzymes were assessed on their ability to catalyze two consecutive hydroxylation steps leading from the germacrene A to the costunolide, the common precursor of sesquiterpene lactones (STLs). The enzymes were functionally characterized in both yeast and Nicotiana benthamiana, and their involvement in STLs biosynthesis was demonstrated. The substrate specificity of CYP71AV9 was also investigated by testing its ability to convert amorpha-4,11-diene, (+)-germacrene D and cascarilladiene to their oxidized products when co-expressed in yeast with the corresponding terpene synthases.

To investigate the distribution and accumulation of the cynaropicrin in plant, we performed metabolic profiling of different tissues extracts: leaves and capitula (receptacles and external bracts) at different developmental stages as well as stems and roots at commercial stage. Cynaropicrin was found to accumulate mainly in leaves, in which, by means of RT-PCR, the highest expression of
CcGAS, CcGAO and CcCOS genes was proven. The direct correlation between cynaropicrin contents and expression of the three genes confirmed the key role played by the latter in controlling sesquiterpenes lactone biosynthesis. Furthermore, through phytochemical analysis of the glandular trichomes extracts, we found out that STLs in globe artichoke accumulates in this specific organ, presumably to avoid self-toxicity.