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Poster Abstract - F.22

ISOLATION AND FUNCTIONAL CHARACTERIZATION OF A cDNA CODING AN HYDROXYCINNAMOYLTRANSFERASE INVOLVED IN PHENYLPROPANOID BIOSYNTHESIS IN *CYNARA CARDUNCULUS* L.

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Cynara cardunculus includes globe artichoke (var. *scolymus* L.), cultivated cardoon (var. *altilis* DC) and their progenitor wild cardoon [var. *sylvestris* (Lamk) Fiori].

Wild and cultivated forms of the species are a source of biopharmaceuticals. The chemical components of the leaves have been found rich in compounds originating from the metabolism of phenylpropanoids which (i) protect proteins, lipids and DNA from oxidative damage caused by free radicals, (ii) inhibit cholesterol biosynthesis and contribute to the prevention of arteriosclerosis and other vascular disorders, (iii) inhibit HIV integrase, a key player in HIV replication and its insertion into host DNA, and (iv) possess antibacterial activity.

The major phenolic compounds in artichoke extracts are di-caffeoylquinic acids (e.g. cynarin) which are present mainly in *Cynara* species, and its precursor chlorogenic acid, one of the most widespread soluble phenolic compound in the plant kingdom.

Tobacco contains an enzyme: hydroxycinnamoyl-CoA:shikimate/quinate hydroxycinnamoyltransferase (HCT), which controls the biosynthesis of chlorogenic acid from p-coumaroyl-CoA and quinic acid.

mRNAs were extracted from globe artichoke leaves and the cDNAs generated by reverse transcription. Degenerate CODEHOP primers were designed on conserved region of acyltransferase protein in order to amplify the HCT cDNA by PCR. The resulting DNA fragments were resolved by agarose gel electrophoresis and a band of 700 bp isolated, cloned into a plasmid and sequenced.

A translated database search (Blast x) revealed high similarity (79% identity and 86% homology) with the tobacco HCT (Accession AJ507825) and global alignment (CLUSTAL W) revealed the artichoke HCT sequence clustering with one of the four main acyltransferase groups (i.e. anthranilate N-hydroxycinnamoyl/benzoyltransferase). After successful full length HCT cDNA isolation by 3' and 5' RACE, the gene was cloned in (pET3a and pET28b) and heterologously expressed in *E. coli*.

Reaction products were identified by HPLC: the expressed enzyme was found to catalyze the synthesis of quinate ester (p-coumaroyl-quinate, an chlorogenic acid precursor) from p-coumaroyl-CoA and quinic acid.

Northern blot assays showed different levels of expression of the artichoke HCT in wild and cultivated forms of *Cynara cardunculus* .