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Poster Abstract – D.20

CLONING AND FUNCTIONAL CHARACTERIZATION OF TWO HYDROXYCINNAMOYLTRANSFERASES INVOLVED IN PHENYLPROPANOID BIOSYNTHESIS IN *C. CARDUNCULUS* L.

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Many secondary metabolites found in plant tissues are of high pharmaceutical interest although they are usually present at low concentrations. Among therapeutic molecules produced by plants the phenolic compounds are the most widespread.

Cynara cardunculus species is source of biopharmaceuticals and its leaf extracts have been widely used in herbal medicine as hepatoprotectors and choleretics since ancient times. In *Cynara cardunculus* extracts the dominating phenolic compounds are di-caffeoylquinic acids (e.g. cynarin), which originate from the metabolism of phenylpropanoids, along with their precursor chlorogenic acid (CGA).

In order to identify new ways of production, it is important to acquire new knowledge on the biosynthetic pathways of exploitable secondary metabolites. The aim of our study was to identify genes encoding acyltransferases involved in caffeoyl quinic acids synthesis.

mRNAs were extracted from globe artichoke leaves and the cDNAs generated by reverse transcription. Degenerated primers were designed with CODEHOP strategy on conserved regions of orthologous HCT (hydroxycinnamoyl-CoA: shikimate/quinate hydroxycinnamoyltransferase) and HQT (hydroxycinnamoyl-CoA quinate: hydroxycinnamoyltransferase) proteins from tobacco, with the objective to amplify in artichoke the HCT and HQT cDNA by PCR. A translated database search revealed high similarity, for two amplicons sequenced, with the tobacco HCT and the HQT of tobacco and tomato, respectively. After successful full length HCT and HQT cDNA isolation, the genes were cloned in plasmids and heterologously expressed in *E. coli* for protein recovery.

For HCT gene, reaction products were identified by HPLC: the expressed enzyme accepted both caffeoyl CoA and *p*-coumaroyl CoA as substrates, with either quinate and shikimate to synthesize caffeoyl quinate (i.e. chlorogenic acid), *p*-coumaroyl quinate, caffeoyl shikimate or *p*-coumaroyl shikimate, depending on the substrates supplied. Moreover variable levels of HCT transcripts (assessed by northern blot assay) were shown among wild and cultivated forms of *C. cardunculus* subspecies. This level of expression was correlated with CGA content, supporting the predicted involvement of HCT in the caffeoylquinic acids synthesis.

Analyses on the reactions catalysed by the second isolated enzyme (putatively HQT) are now in process, to complete the investigation on the role of these acyltransferases in phenylpropanoids biosynthesis.