Proceedings of the 53rd Italian Society of Agricultural Genetics Annual Congress
Torino, Italy – 16/19 September, 2009
ISBN **978-88-900622-9-2**

Oral Communication Abstract – 3.04

NOVEL HYDROXYCYNNAMOYL-QUINATE TRANSFERASE (HQT) GENES ACT IN A NETWORK FOR THE SYNTHESIS OF CHLOROGENIC ACID IN ARTICHOKE

SONNANTE Ga.*[*], D'AMORE R.*, LUO J.**, BLANCO E.*, PIERRI C.L.***, MARTIN C.**

*) Institute of Plant Genetics - National Research Council (CNR), Via Amendola 165/A,
70126 Bari, Italy - [#]gabriella.sonnante@igv.cnr.it
**) John Innes Centre, Norwich Research Park, Colney, Norwich, NR4 7UH, UK
***) Pharmaco-Biology Department, University of Bari, Via Orabona, 4, 70126 Bari

hydroxycynnamoyl-quinate transferase genes, chlorogenic acid, enzyme specificity, modeling, docking

Most of the artichoke beneficial properties, such as antioxidant, hepatoprotective, coleretic, and hypocolesterol activity, are attributed to polyphenols, a class of compounds with beneficial effects on human health. These secondary metabolites include hydroxycinnamates, particularly caffeic acid, chlorogenic acid (CGA), di-caffeoylquinic acids, and flavonoids (e.g. luteolin). Chlorogenic acid is the most abundant polyphenol in artichoke. In plants, the synthesis of this compound is supposed to occur following three possible metabolic routes, and the enzyme hydroxycynnamoyl-quinate transferase (HQT), belonging to the acyl transferase BAHD family, is reported to be involved in the last steps of this biochemical pathway. In the present contribution, we report on the isolation and characterization of two artichoke full-length *hqt* and *hqt2* cDNA sequences. Genomic organization of the genes revealed that they contain two exons and one intron. The obtained sequences showed a high level of similarity to homologous genes from other plant species, particularly tobacco and tomato. Heterologous expression of both transcripts in *E. coli* allowed the biochemical characterization of the two artichoke HQTs. Kinetic analyses indicate that both enzymes show a much higher affinity towards quinate compared to shikimate, however the preferred acyl donor seems to be different for the two enzymes.

Comparative models of artichoke HQT, HQT2 and HCT (as negative control) were built based upon the available crystallized structures of two BAHD enzymes (PDB codes: 2e1t and 2bgh), by using the computer application MODELLER. A critical determinant in the accuracy of comparative modelling is the quality of the pair-wise alignment between the investigated proteins and the crystallized templates. The structural properties of the HQT, HQT2 and HCT 3D models with the best energy function were evaluated by using the WHATIF biochemical/computational tools. Potential binding sites of HQT, HQT2 and HCT were predicted to investigate them with docking studies. AUTODOCK was employed to evaluate the fit of potential substrates within the proposed binding sites.

In order to assess levels of expression of the two isolated genes, real time PCR was carried out for different plant organs, including flower heads and leaves, and developmental stages. Results revealed that the two genes have a different behaviour and are differentially expressed in artichoke plant parts/stages.

The overall results suggest that the two genes can complement each other in the synthesis of CGA in artichoke, according to the plant tissue and the physiological stages considered.

A better understanding of how the artichoke genes involved in the pathway leading to CGA and di-caffeoylquinic acids production interact is important to develop agronomic, genetic, or biotechnological tools for higher production of biologically active compounds in artichoke.