

HPPR GENE IDENTIFICATION IN *SALVIA OFFICINALIS* CELL CULTURES FOR THE PRODUCTION OF ROSMARINIC ACID

PISTELLI LA.* BARBERINI S.*, RAFFI D.** , LEONARDI M.*** , BERTOLI A.*** ,
PISTELLI LU.*** , RUFFONI B.**

*) Department of Biology, University of Pisa, Via Mariscoglio 34, 56124 Pisa (Italy)

**) CRA-FSO Research Unit for Floriculture and Ornamental Species, Sanremo (Italy)

***) Department of Farmaceutical Sciences, University of Pisa (Italy)

Hydroxyphenylpyruvate reductase (HPPR) gene, Salvia officinalis, Rosmarinic acid, cell cultures, nutraceutical

Several bioactive substances have been recently taken into consideration for their applications as food preservative, nutraceuticals and for pharmaceutical purposes due to functional properties as antioxidant and antimicrobial compounds. Plant species belonging to Lamiaceae and Boraginaceae are very rich in rosmarinic acid (RA), an ester of caffeic acid with 3,4-dihydroxyphenyl-lactic acid. The presence of RA in medicinal plants, herbs and spices has beneficial and health promoting effects, for its interesting biological activities, e.g. antiviral, antibacterial, antiinflammatory and antioxidant agent. RA is supposed to act in plant as a defence compound. The biosynthesis of RA has been recently defined: HPPR (hydroxyphenylpyruvate reductase) is considered the first specific enzyme and represents the key-enzyme responsible for this metabolic pathway.

In this work *Salvia officinalis* L. cell cultures were studied in order to 1) analyse the production of antioxidants and RA, 2) isolate a cDNA and identify the hydroxyphenylpyruvate reductase (HPPR) gene, 3) evaluate the use of the HPPR gene as molecular marker for the controlled production of the metabolite of interest.

A specific *S. officinalis* cell line was selected for the high antioxidant capacity of its hydroalcoholic extract, which was characterized by a very high content of RA. The antioxidant total capacity (DPPH test) and the total RA content were evaluated during the cell growth curve related to the developmental phase.

cDNA obtained from *Salvia officinalis* L. cell cultures was then analysed by PCR, using pairs of specific and degenerate primers built on the basis of information in GenBank. The results of the sequencing of PCR products have been successful in aligning with the software BLAST.

cDNA partial sequence of the (HPPR) gene was then isolated from *Salvia officinalis* cell cultures (GenBank: EU924744.1). HPPR gene expression is correlated to the production of RA in cell cultures.

So far the identification of the homologous cDNA sequence of the HPPR enzyme in suspension cultures of *Salvia officinalis* can increase the knowledge of RA biosynthesis in this plant and act as a marker of the antioxidant activity.