

## TISSUE CULTURE OF TRANSGENIC OLIVE OVEREXPRESSING THE TOBACCO OSMOTIN GENE, A PR - PROTEIN WITH INTERESTING BIOLOGICAL ACTIVITY

LUPO M.\*, MASSA S.\*\*, VENUTI A.\*\*\*, PAOLINI F.\*\*\*, PAGLIARELLO R.\*\*,  
CRISTOFORI V.\*, RUGINI E.\*, CIAFFI M.\*\*\*\*, PAOLACCI A.\*\*\*\*, SILVESTRI C.\*

\*) University of Tuscia, Department of Agriculture and Forest Sciences (DAFNE), Via San Camillo de Lellis snc, 01100 Viterbo, Italy

\*\*) ENEA, Italian National Agency for New Technologies, Energy and Sustainable Economic Development. Department for Sustainability, Biotechnology and Agro-Industry Division – Biotec Laboratory; Casaccia Research Center, Rome, Italy

\*\*\*) HPV-UNIT-UOSD Tumor Immunology and Immunotherapy, IRCCS Regina Elena National Cancer Institute, Rome, 00144, Italy

\*\*\*\*) University of Tuscia, Department for Innovation in Biological, Agro-food and Forest systems (DIBAF), Viterbo, Italy

*osmotin, plant tissue culture, cytotoxic activity, Olea europea L., cervical cancer*

Osmotin is a plant multifunctional protein responsive to many biotic and abiotic stresses, including osmotic stress. Several studies demonstrate that this protein shows functional homology with adiponectin, a human hormone with antidiabetic, antiatherogenic, anti-inflammatory and anti-tumor effects. Chemical synthesis of adiponectin is considered difficult because of many post-transcriptional and post-translational modifications. For this reason, osmotin may be a valid candidate for drug therapy, as it is produced in plants and its action in mammals has already been evaluated by both *in vitro* and *in vivo* studies on atherosclerosis and neurodegenerative diseases. Genetic engineering combined with plant tissue culture makes it possible to create fast, cheap and environmentally friendly production platforms for compounds that can be used for human health. In this study, explants of *Olea europea* cv. Canino, transgenic for the tobacco osmotin gene were studied as a potential source for the bioaccumulation of osmotin. Firstly, the presence of the transgene was confirmed via PCR analysis, then western blotting analysis verified the

effective bioaccumulation of the protein. Total soluble protein (TSP) extracts, containing osmotin, were then tested on non-tumor cells (HaCat cells) to evaluate the cytotoxicity via MTT assay. Results showed that these extracts are not toxic for normal cells, except at high doses. On the contrary, the same test conducted on CaSki cancer cell line derived from Human Papillomavirus cervical carcinoma showed that TSP extracts containing osmotin have antiproliferative activity even at low doses.

These preliminary experiments show osmotin effective anti-proliferative activity on a specific human tumor cell line model, without exerting any cytotoxicity on non-tumor cells, except at high doses. Nevertheless, this study represents a starting point for the overproduction of osmotin in different plant culture formats such as hairy roots or cell suspension culture, that are very suitable for industrial "scale-up", to be tested in different models of chronic pathologies.