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Poster Abstract – D.12

ANALYSIS OF GENETIC AND EPIGENETIC EVENTS DURING GROWTH AND SENESCENCE IN GRAPE CELL CULTURES

ZOTTINI M.*, RODIO S.*, RUBERTI C.*, LO SCHIAVO F.*, VAROTTO S.**

*) Department of Biology University of Padova, Via U. Bassi 58B, 35121 Padova
**) Department of Environmental Agronomy and Plant Production, University of Padova, Viale dell'Università 16, 35020 Legnaro (PD) (Italy)

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Senescence is a highly-regulated process, difficult to characterise in planta because of its complexity. By using grapevine cell cultures as experimental system, we are trying to identify genes involved in the regulation of senescence. In view of the difficulties encountered in studying the entire plant, a tree with a long reproductive cycle, the use of cell cultures is instrumental for reaching our goal, but the results acquired in cell cultures will be applied to processes in planta.

Cell cultures represent an ideal system for the determination of the effects of epigenetic modifications on growth and ageing. DNA methylation and post-transcriptional modifications of histones represent the best characterized epigenetic regulation mechanisms in plants. Cytosine methylation occurs in CpG sequence context both in higher plants and animals, but plants differ from animals for significant levels of methylayion at symmetric CpNpG. Two essential roles have been ascribed to DNA methylation: defending genome against transposons and regulating gene expression. The N-terminal and C-terminal tails of histones are subject to post-translational modifications, such as acetylation, methylation, phosphorilation, ubiquitination and others. These covalent modifications are able to modulate chromatin structure and transcription, both directly causing structural changes to chromatin and indirectly recruiting protein complexes that can read these marks and elicit a response. In particular we are studying the involvement of cytosine methylation and histone modifications on growth and aging and their relationships with the transcription and/or silencing of specific set of genes. Initially, genes modulated during cell growth and senescence have been identified in a Köber variety cell line. Among these genes, ten of them were analysed in more details. Subsequently, the pattern of expression of each gene was compared with its methylation status through a DNA McrBC digestion followed by a PCR analysis.

Subtle changes in chromatin structure might be required for fine-tuning of gene expression. For this reason we analysed the heterochromatic histone H3 methylation marks in growing cell cultures by using specific histone methylation antibodies (H3K4,H3K9,H3K27) in an immuohistochemical assay performed on nuclei of cultured cells and in westernblot analyses perfomed on proteins extracted from cells at different physiological stages. Other histone marks will be analyzed in further experiments. Our future aim is to develop epigenetic protocols, such as ChIP and ChIP-seq, for elucidating the roles of heritable traits that do not depend on the primary sequence of DNA but contribute to regulate senescence in grapevine.