EPIGENETIC AND MOLECULAR STUDIES DURING AGING OF AGROFRESTRY SPECIES

Rodríguez R.[1,2], Berdasco M.[1], Diego B.[1], Hasbún R.[1,3], Valledor L.[1], Testillano P.[4], Risueño C.[4], Fraga M.[1,5], Cañal MJ.[1,2]

[1]Lab. Fisiología Vegetal, Dpto. B.O.S., Facultad de Biología, Universidad de Oviedo. C/ Catedrático Rodrigo Uría s/n. E-33071, Oviedo, España.

[2]Instituto de Biotecnología de Asturias (asociado al CSIC). E-33071, Oviedo, España.

[3]Lab. de Biotecnología Forestal, Facultad de Ciencias Forestales, Universidad de Concepción. Victoria 631, Casilla 160 C, Correo 3. Concepción. Chile.

[4]Centro de Investigaciones Biológicas. C/Ramiro de Maeztu 9 E-28040, Madrid, España.

[5]Lab. Epigenética del Cáncer, Programa de Patología Molecular, Centro Nacional de Investigaciones Oncológicas (CNIO). C/Melchor Fernández Almagro, N°3. E-28029, Madrid, España.

Optimization of forestry breeding programs can not be restricted to genetic markers screening. It is essential to determine the changes in gene expression profiles that are associated to ageing and rejuvenation, and consequent changes in physiological pathways. Epigenetic mechanisms such as DNA methylation, and histone acetylation are main topics of our current research carried out in processes of maturation, ageing-rejuvenation and phase-change of agroforestry species. Global DNA methylation, site-specific methylation and *in situ* methylation were analyzed in juvenile and mature individuals from selected and not selected clones as in tissues proceeding from different organs at different states of development/differentiation. We have also studied the *in situ* localization of methylcytosines in cells and tissues of meristematic and differentiated leaf areas from juvenile, mature vegetative and mature reproductive individuals by inmunofluorescence with specific antibodies. The methylation status of specific genes promoters were studied on genes related with the biosynthetic pathway of the polyamines and, after bisulfate modification and sequentiation, we can establish a relation between the expression/repression of these genes and the specific methylated/unmethylated patterns of their regulatory elements. Structural analysis of samples at different maturation stages has been accomplished to characterize changes in the organization of cells and tissues and to define specific developmental stages for further inmunolocalization studies. Antibodies against acetylated forms of histones H3 and H4 were used for inmunofluorescence and results were analyzed under confocal microscopy. Cyclin b1 was also studied as a marker for cell cycle specific phases (G2-M) in order to analyze patterns of localization/distribution of proliferating/cycling cells in leaves with different state of differentiation/maturation. The specifity of the antibodies for the plant material was achieved by “Western blotting” which also informed about differences in the histone acetylation and cyclin b1 expression among the tissues under study. Differential Display – RTPCR analyses allows the comparison of expression patterns in different tissues revealing some presumptive messengers with age- and reinvigoration-dependent expression. Homology inferences have revealed the identity of these candidates Northern hybridisation has demonstrated the association between the decrease of expression level of *Arabidopsis thaliana* RPT5 proteosome ATPase homologue in *Pinus radiata* and reinvigoration in vegetative terminal buds. The validation of RPT5 as a useful ageing molecular marker is being performed by expression studies in juvenile and adult selected offsprings and in commercial plantations, as well as in macrograft reinvigoration experimental systems. No variation in proteasome components or activity had been reported in reference to tree ageing or reinvigoration. However, there are some references about proteasome involvement in differentiation and maturation processes in other plants. Proteosome activity is also related to the modulation of cytokinin and auxin responses, so the queues obtained about the variations of the expression levels of proteasome components are in accordance with previously reported

specific ageing and maturation auxin and cytokinin contents that were validated as putative physiological markers. *Pinus radiata* D. Don and *Castanea sativa* Mill were selected as experimental plant material