Se aplicó un análisis de componendes principales a todos los spots, permitiendo el PC2 la separación de las muestras en dos grupos. Teniendo en cuenta los análisis uni y multivariables se seleccionaron para su identificación aquellos los 50 spots que mostraron un p-valor más bajo en el caso de la prueba T, y aquellos con coeficientes de correlación mayores de 0.85 para los componentes principales 1-3.

La identificación de los spots diferenciales permitió describir por primera vez en helechos las rutas metabolicas cuya expresión varía en relación con el desarrollo sexual dependiente de la acción del anteridiógeno.

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Proteome regulation and epigenetic code during *Pinus radiata* needle maturation

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Needle differentiation is a very complex process which leads to the formation of a mature photosynthetic organ. This fact implies important changes in protein accumulation and gene expression which must be regulated, amongst others, by epigenetic mechanisms. We have compared some epigenetic modifications (DNA methylation, Histone H3K4^{3m}, Histone H3K9^{3m} and acetylated Histone H4) present in immature (1 month old) and mature (12 month old) *Pinus radiata* needles (Figure 1a), determining a tissue specific DNA methylation (Figure 1b) and the differential expression levels of histone epigenetic marks, being the levels of acetylated Histone H4 and Histone H3K4^{3m}, associated to gene expression, higher in immature needles whereas the Histone H3K9^{3m} was only found in mature needles (Figure 1c). This could be explained by the fact that chromatin needs to be altered and restructured during the differentiation to regulate gene expression [1].

To determinate which genes and proteins showed as differential during needle development

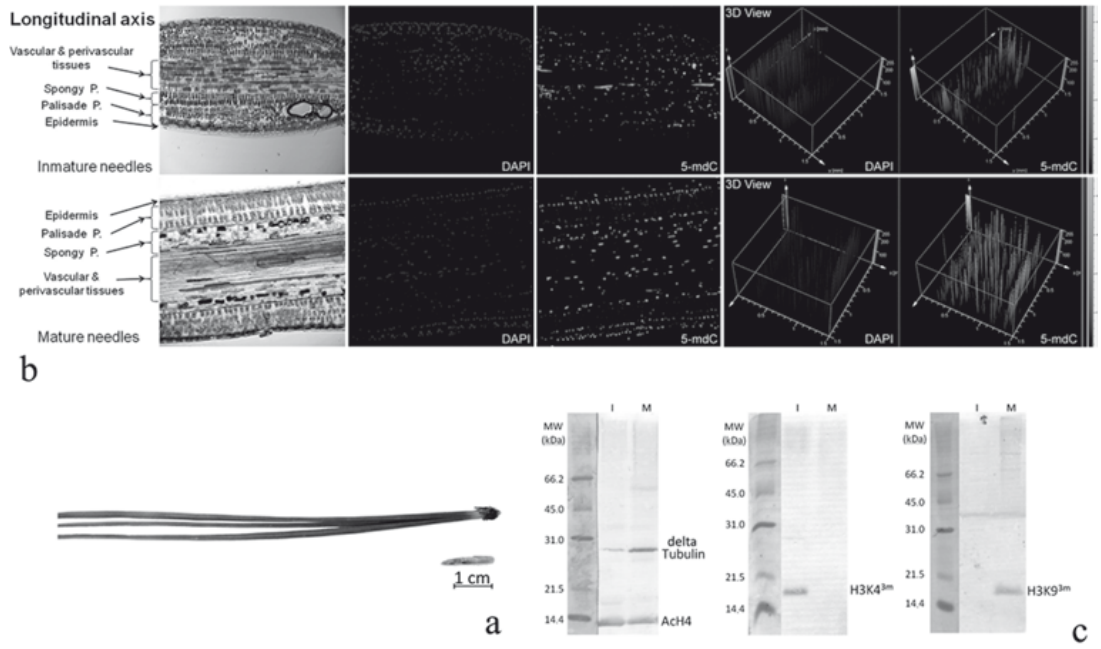

Figure 1. *a) Mature (top) and immature (bottom) needle fascicles. b) Immunodetection of 5-mdC. In 3D view the intensity of each fluorescence point is represented, larger vertical bars indicate higher fluorescence intensity. c) Representative blots showing the quantification of AcH4, H3K4^{3m} and H3K9^{3m} on protein extracts from immature (I) and mature (M) scions. The left lane corresponds to Coomassie stained MW standards.*

we have also characterized and compared proteome and transcriptome of immature and mature needles. Immature needles are characterized by a low tissue differentiation and high morphogenetic capability whereas mature needles are a specialized photosynthetic organ, which do not show morphogenetic capabilities. 2-DE profiles determined following a previously described protocol [2], showed variations in the relative abundance of 280 spots from a total of 856 that were studied whereas transcriptomic analyses (substractive library building and determination of differential expression by macroarray and real time PCR) resulted in the description of 176 differentially expressed genes in immature and mature needles. The joint analysis of proteomic and transcriptomic data that was performed provided a broad overview of differentially expressed genes and proteins associated with needle maturation. Some spots and genes related to photosynthesis and oxidative phosphorylation were overexpressed in mature needles. On the other hand immature needles were characterized by the overexpression of biosynthesis-, cell division- and differentiation-related proteins [3]. A joint data analysis of proteomic and transcriptomic results provided a broader view over differentially expressed pathways associated with needle maturation. Energy metabolism pathways, with photosynthetic and oxidative phosphorylation related proteins and genes, were overexpressed in mature needles. Amino acid metabolism, transcription and translation pathways were overexpressed in immature needles. Interestingly, stress related proteins and defense mechanisms were characteristic of immature tissues, a fact that may be linked to the trees' need to defend the needles in young stages or the higher growth rate and morphogenetic competence exhibited by this tissue [3].

From these functional groups we have selected five genes related to photosynthesis (*RBCA*), regulation of gene expression (*MSI1, CSDP2*), leaf elongation (*CYP78A7*) and stress (SHM4) to further investigate its specific DNA methylation. All of these genes showed differential histone modifications, detected by Chromatin Immunoprecipitation, furthermore *CYP78A7* and *CSDP2* showed a sequence rich in CpG in its promoter and first exon. Specific DNA methylation patterns related to tissue differentiation were found for *CSDP2* [4]. This is the first description of a specific gene regulation based on epigenetic mechanisms in conifers.

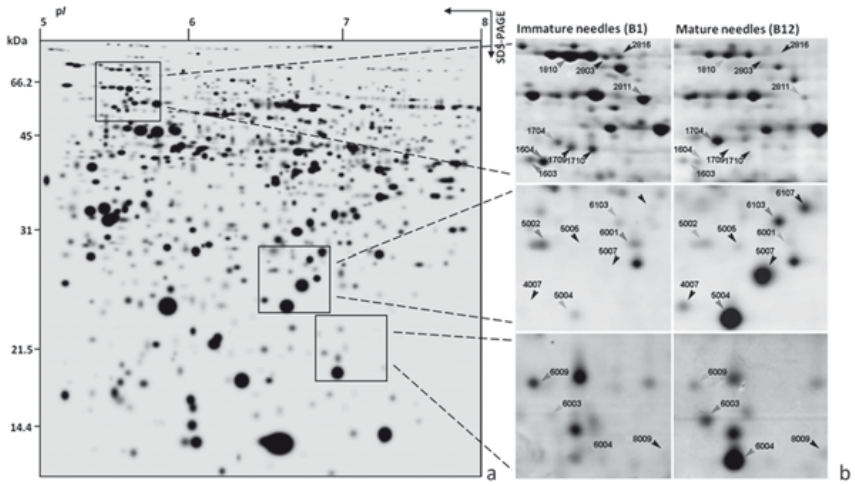

Figure 2. *Master gel combining spots of B1 and B12 protein extracts (a). Three sections of the 2-DE gels showing two representative B1 and B12 gels (b). Red and green arrows up and down accumulated spots, respectively, while black arrows point spots only detected in one kind of protein extract.*

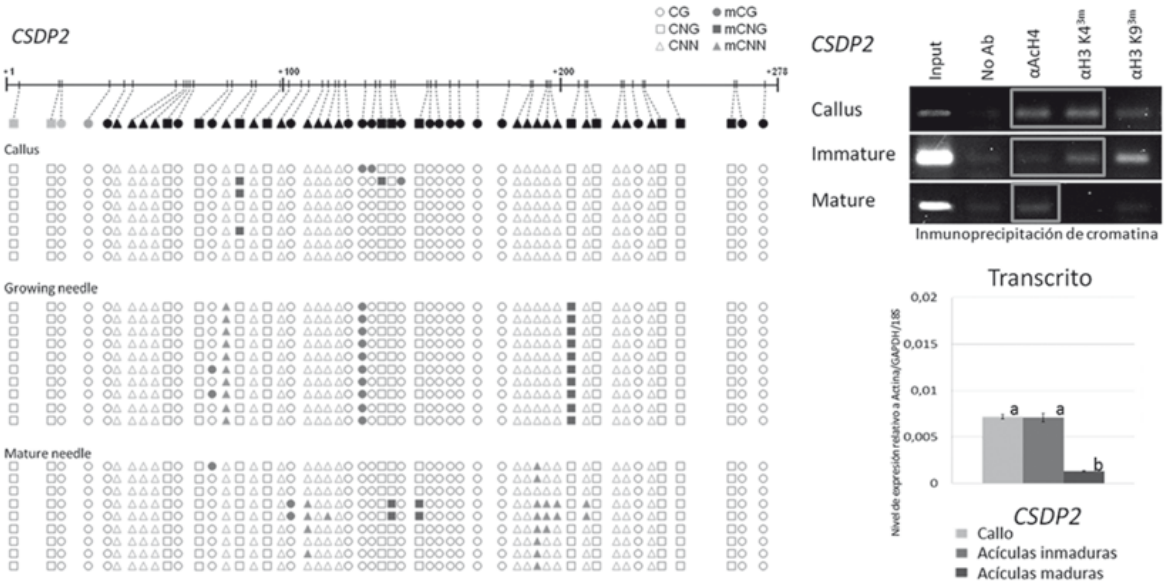

Figure 3. *CSDP2 showed a differential methylation pattern of cytosines. The callus tissue, without any methylated cytosines, showed the highest expression level of this gene and the higher proportion of AcH4 and H3K4^{3m} (all epigenetic marks associated with gene expression). One of these marks, H3K4^{3m}, was lost in mature needles, the tissue which showed the lowest expression level. (Valledor et al., in preparation)*

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