ANALYSIS OF THE HOLM OAK (QUERCUS ILEX SUBSP. BALLOTA) POLLEN PROTEOME BY USING NLC-MS/MS

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Our groups are carrying out a research project aimed at studying natural variability in Holm oak (*Quercus ilex* subsp. *ballota* (Desf.) Samp.), with some results already published (Jorge et al. 2005, 2006, Echevarría et al. 2009, Valero et al. 2010). The high variability found in the leaf 2-DE protein profile, even within the same tree and depending, among other factors, of the leaf orientation, position, sampling time, developmental stage, and environment, encouraged us to use other organs such as fruit and pollen for such as purpose, as they are suposed to have a less variable proteome. Biodiversity in plant species by using proteomics and simple experimental material such as pollen have a number of advantages if compared with other approaches and tissues. In the present work, a gel-free/label-free approach has been used to characterize the holm oak pollen proteome.

Proteins were extracted from pollen using the TCA-acetone-phenol protocol (Wang et al. 2006), as reported in Maldonado et al. (2008). Total protein extracts were digested with trypsin, tryptic peptides were separated by nano-HPLC and analyzed in an Orbitrap mass spectrometer.

This analysis has allowed the identification of 529 protein species belonging to several functional categories, being the proteome dominated by enzymes of different pathways, protein fate, cell structure, disease, and defense. However, as typical for orphan tree species (Jorrín et al. 2009) and as experimented before in our previous studies on *Q.ilex* (Jorge et al. 2005, 2006, Echevarría-Zomeño et al. 2009) the absence of *Quercus* spp. DNA, proteins or ESTs entries in databases made the protein identification difficult as well, even when they have been properly extracted, separated and detected.

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