

PROTEOMICS AS AN APPROACH TO STUDYING VARIABILITY IN HOLM OAK (*QUERCUS ILEX* SUBSP. *BALLOTA* [DESF] SAMP) ANDALUSIAN POPULATIONS

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Our groups are carrying out a multidisciplinary research project on holm oak (*Quercus ilex* subsp. *ballota* [Desf] Samp) and other forest tree species of interest within the Andalusian region. We aim: i) to characterize the different catalogued provenances; ii) to study differential population responses to biotic and abiotic stresses; iii) to select elite trees to be used in reforestation programmes; and, iv) to optimize *in vitro* propagation protocols. For these proposals we are using different approaches, including classic biochemistry, proteomics, and genomics. Previously, differences in the 2-DE protein pattern between population and changes in response to drought stress were studied on leaf tissue (Jorge et al. 2005, 2006). Due to the great variability found in the leaf proteome, even within the same tree, it was almost impossible to establish clear differences between provenances, and we therefore decided to move to a different organ, that, like the seed, has a more stable proteome. This communication deals with the specific objective of analyzing variability in holm oak (*Quercus ilex* subsp. *ballota* [Desf] Samp) Andalusian populations by comparing the 1- and 2-DE protein profile of mature seeds. Seed proteins (10 populations, 5 trees per population) were TCA-acetone extracted and subjected to both 1- (SDS-PAGE) and 2-DE (IEF/SDS-PAGE). Coomassie-stained gel images were captured with a GS-800 Calibrated Densitometer (Bio-Rad) and analyzed by PD-Quest software. The 1-D banding pattern was highly reproducible among trees within populations, founding 7 polymorphic bands. 2-DE analysis was limited to just four out of the ten populations sampled. Qualitative differences were found for 74 of the around 352 spots detected. Some of the differential bands/spots have been identified after MS analysis, with most of them corresponding to legumins, a type of globulin described in different *Quercus* species. Principal component analysis and dice coefficient have been used to determine the most discriminate bands/spots and the genetic distances between provenances.

1. Jorge I., Navarro R.M., Lenz C., Ariza D., Porras C., Jorrin J. 2005. The holm oak leaf proteome: Analytical and biological variability in the protein expression level assessed by 2- DE and protein identification tandem mass spectrometry de novo sequence similarity searching. *Proteomics*, 5:222-234.
2. Jorge I., Navarro R.M., Lenz C., Ariza D., Porras C., Jorrin J. 2006. Variation in the holm oak leaf proteome at different plant developmental stages, between provenances and in response to drought stress. *Proteomics*, 6:207-214.