CHARACTERIZATION OF CORK OAK (QUERCUS SUBER L.) GENETIC RESOURCES FOR TREE IMPROVEMENT

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Summary
Cork Oak (*Quercus suber* L) is one of the most important forest species in the Mediterranean area. In this study, mature leaves of 53 trees from four origin regions were analysed using Inter Simple Sequence Repeat (ISSR) markers since 32 trees were analysed using Simple Sequence Repeat (SSR) markers. Despite the most of trees from each region were localized in the same UPGMA group, it is possible to observe the high variability found in the samples, showing the importance of these trees in future breeding programs.

Keywords
Cork oak, ISSR, SSR, origin regions, genetic analysis

Material and methods
Fifty three trees of *Q. suber* L from four Spain origin regions: Norte de Cáceres- Salamanca (R1), Sierra de San Pedro (R2), Montes de Toledo (R3) and Sierra Morena Occidental (R4) were sampled. These 53 trees, selected by high production and cork quality, were analysed by ISSR since 32 of them were analysed by SSR markers.
DNA from mature leaves was isolated using the modified Doyle & Doyle protocol (1990). Microsatellite and ISSR markers were used to characterize each individual. Three *Q. petrea* loci were amplified with the primers QpZAG15, QpZAG46, QpZAG110, chosen by their polymorphism. The amplification conditions reaction was prepared as described by Gómez *et al* (2001). Fluorescent labelled PCR products were separated and analysed on a semiautomated sequencer. Six ISSR markers (set #9 University of British Columbia Laboratory - UCB, Cánada) were chosen by their clear and reproducible banding pattern. Amplification results were visualized through agarose (1.5%) gel electrophoresis and ethidium bromide staining. Bands sizes were estimated by comparing with a marker ladder (100 bp ladder, Pharmacia).
Microsatellite amplifications were expressed as allele size, in base pairs. A similarity matrix of allele presence (1) and absence (0) was calculated by the DICE's coefficient, for ISSR the matrix was calculated by the "Simplematching" coefficient and the dendrograms were obtained by the UPGMA method from NTSYS-pc package (Rohlf, 1998). The observed heterozygosity and the power of discrimination for each *locus* were calculated as indicate Kloosterman *et al* (1993). PIC (Polymorphic Content Index) was calculated following Raina et al. (2001).

Results
The average number of different alleles per microsatellite locus was 4 (QpZAG15 and QpZAG46) and 16 (QpZAG110). On each locus at least, one main allele could be identified and should be the more ancient allele (Charlesworth et al, 1994). The ISSR analysis resulted in near 90.6% of polymorphic bands. The number of bands for each ISSR marker range

between 11 and 22, with an average of 14.2.The allelic diversity detected by microsatellite markers is higher than that earlier reported in other Quercus spp and the allele range in select trees was found similar to those observed in previous works (Steinkellner et al., 1997; Hornero et al., 2001), despite the low number of trees and microsatellite loci analysed in this study. All analysed markers allowed differentiating all the tested individuals. Heterozygosity average value was 0.703 and the discrimination power between genotypes was 96.86% by SSR markers since ISSR bands showed a high discrimination power (PIC) that ranges from 0.15 to 0.5. The clustering of the individuals indicates that 57% of trees from Sierra Morena Occidental region (R4) were localized on the first group. The second group has a high percentage (67%) of trees from Montes de Toledo region (R3). A third group consist only of two individuals from different origin regions. Despite the most of trees from each region were localized in the same groups, is possible to observe the high variability found in the samples, showing the importance of these trees in future breeding programs.

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