**SUMMARY**

The cork oak (*Quercus suber* L.) is one of the most important Mediterranean forest tree species. The cork oak spreads over the western and coastal region of the Mediterranean area, under an Atlantic influence, occupying the biggest areas in Portugal and Spain. Andalusia, Extremadura and Catalonia have the largest cork oak forests in Spain. Cork oak's main product is cork, which can be stripped from the tree without severe damage at regular intervals. Hence it is a renewable raw material for the production of stoppers in wine industry, the main use at present. This production determines the management of the cork oak stands. Besides its economic value, the cork oak tree has also an important social and ecological value to Spain, being part of "dehesas", which are forest and grazing land systems of great importance for rural development. Nowadays, cork oak stands are endangered because of several causes like forest fire, excessive grazing, replacement by crops, low natural regeneration, losses by cork oak decay ("seca") and commercial competition with cork substitutes. The cork oak tree is a recalcitrant species for seed conservation and morphogenic ability. Therefore, developing vegetative propagation technologies for conservation of genetic resources and improvement of the species are of paramount importance.

Somatic embryogenesis is considered the best tissue culture-based way of plant regeneration in forest biotechnology. The present work studies some aspects of somatic embryogenesis for clonal regeneration of mature cork oak trees. This thesis is divided in several chapters, related to the consecutive phases of the process: induction of somatic embryogenesis, multiplication by recurrent embryogenesis and somatic embryo conversion. The application of AFLP markers to test the donor trees for genetic uniformity has also been studied.

Expanding leaves were used as initial explants to induce somatic embryogenesis. They were excised from epicormic shoots, which were forced to flush from pieces of branches taken from the crown of mature cork oak trees. The induction of somatic embryogenesis was achieved on media supplemented with 1-naphthaleneacetic acid and 6-benzylaminopurine, first at high concentration and then on a secondary medium at lower concentration. Most somatic embryos were obtained in a third phase, on the same medium lacking plant growth

regulators. Embryos seemed to arise directly on the tissue surface. Embryogenic masses were formed by secondary embryogenesis, starting a very productive recurrent process, without decline in their multiplication ability for years. The presence, concentration and exposure time to plant growth regulators influenced the embryogenic response. The best response was achieved with 50 µM NAA plus 10 µM BAP in the primary induction medium, and 30 days culture on this medium. Only expanding leaves that were less than 15 mm length gave somatic embryos. Therefore, the existence of a developmental window that could be more prone to induction, similar to the described for the induction in immature cork oak zygotic embryos, is suggested. The frequency of induction of somatic embryogenesis was also influenced by genotype, harvest time and their interaction. The genetic control on the induction process was confirmed: the narrow sense heritability of the ability of cork oak leaves to give somatic embryogenesis was $h^2_i$ =0,41, indicating some degree of additive genetic control on this trait. However no significant effect of geographical provenances was found, the individual effect being more remarkable.

Cork oak embryogenic cultures could be stored at low temperature for four months without loss of their proliferative ability, which permitted to reduce the maintenance costs by increasing subculture periods. Genotype influenced the multiplication of embryogenic lines as well as the conversion of somatic embryos into plants. The degree of maturation of somatic embryos, in terms of size and fresh weight, did not influence their frequency of germination, although affected the survival of the somatic seedlings that they gave rise. A field trial with somatic seedlings from somatic embryos induced from selected cork oak trees and from their half-sib progenies, conjointly with plants from acorns of the same families, was established. Survival and initial growth were better for acorn-derived plants than for somatic seedlings, reflecting the initial advantage of the former due to their higher reserves in cotyledons. The somatic seedlings from adult trees grew better than the somatic seedlings from the half-sib progenies, and they reached the same vigour than zygotic-derived plants very soon, showing the better genetic potential of this vegetative progeny of the selected trees. The efficiency of the defined protocol to clone mature cork oak trees, was confirmed producing plants from all the selected trees, and even from a singular, endangered tree from the Minorca Island, as an example of the application of this technique for the conservation of genetic resources.

AFLP markers are very sensitive to detect changes in the DNA, but caution should be taken because the technique may give some inconsistencies. Up to 3.8% polymorphisms were

considered inconsistencies, and they were related to genotype. When analysing possible intra-individual variability, most of the genotypes showed percentages of polymorphism lower or equal than the considered “error” of the technique. However, up to 7.1% polymorphism was found in one tree among positions. Therefore, pre-existent variability within the donor plants cannot be discarded when performing early testing of somaclonal variation.