

Área Científica **Florestal**

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Título Caracterização do Desenvolvimento Reprodutivo de Quercus suber

Programa

FCT

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Projetos de I&D em todos os Domínios Científicos

Instituição Líder Universidade do Minho

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Parceria

UM	Universidade do Minho	Nacional
ISA	Instituto Superior de Agronomia	Nacional
INIAV	Instituto Nacional de Investigação Agrária e Veterinária, I.P.	Nacional
BioFIG	Centro de Biodiversidade, Genómica Integrativa e Funcional	Nacional

Equipa

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Resumo

The cork oak (*Quercus suber*) forest (the "montado") is a unique resource for Portugal, due to its ecological and socio-economic significance. Recently, a Portuguese Cork Oak ESTs Consortium has produced and sequenced representative ESTs from cork oak. In the current proposal, researchers from the Consortium got together in an initiative to validate the results obtained from the analysis of some of these ESTs libraries and provide, what will be, the first study of molecular characterization of flower induction, female and male flower development and fertilization in a *Quercus* tree species.

Reproductive development is one of the most important stages of the life cycle of a plant. The molecular mechanisms that systematically induce flowering, determine floral organ identity and control fertilization are amongst the molecular genetic developmental pathways better understood in model species [rev 1, 2], and many parallelisms have been found in non model species.

The main objective of this project is to take advantage of the information gathered in our previous project, in which ESTs libraries of female and male *Q. suber* flowers in different development stages were produced, and to contribute to the understanding of potential molecular mechanism that might be involved during reproductive development of this species. Montados are usually naturally regenerated being, therefore, dependent of seed production. However, natural regeneration can be conditioned by several biotic and abiotic factors of the forest environment what might condition the amount of seeds produced. The knowledge of *Q. suber* reproductive biology (flowering and fertilization) is essential to understand the molecular mechanisms of seed production and identify the natural constrains affecting the reproductive success of this species.

Quercus suber is a protandrous monoecious species. Female flowering buds appear in spring, whereas male flowers occur in early spring and autumn. At anthesis, female flowers are not completely formed. Ten to twelve days after pollination the pollen tube growth is arrested, and its growth is only resumed around 3 months after pollination. So far, no molecular characterization of the mechanisms of reproductive development has been reported in any *Quercus* species.

In this project, a full description of the cork oak flower and fruit phenology, in which climatic conditions, flower morphological analysis and corresponding gene expression data are combined, will be achieved. CV-INRB has been studying the reproductive behaviour of this species for many years in controlled stands in different geographic locations. During this project, environmental data will be collected and different phenological traits will be monitored. In parallel, a complete morphological characterization from pre-inductive flowering stages until fruit setting will be accomplished through a combination of different microscopic techniques.

Another objective is to utilize the available female and male flower ESTs libraries to mine information of potential genes involved in flower development. Transcript sequences found specifically in flowering tissues, differentially expressed in male and female flowers and homologous of genes known to be involved in reproductive development in model species will be obtained. This will be achieved with the expert advice of our bioinformatics consultant JL-IGC. Then, the analysis of spatial and temporal expression of these genes in tissues collected during different flower phenological stages by qRT-PCR and RNA in situ hybridization will be accomplished.

Cork oak has a long progamic phase [4] during which important interactions between the male gametophyte and the pistil occur. This delayed fertilization has been associated to specific epigenetic marks patterns [5]. Arabinogalactan proteins (AGPs) are known to be involved in pollen-stigma interactions and are believed to provide recognition signals and directional guidance for the pollen tube to the embryo sac [6]. Therefore, to understand the cellular events during the delayed fertilization process, a dynamic map of epigenetic marks and AGPs distribution along the reproductive processes in *Q. suber* will be established.

The present team gathers several Portuguese labs, with different expertises to embrace this integrative study. The research team, led by the PI that has been working in flower induction pathways and floral organ development (MC-UMinho), also has expertise in pollen development and fertilization (LM-ISA, IM-FCUP, SC-FCUP). We also count with valuable advice of two consultants, one in bioinformatics (JL-IGC), and a world expert in flower development (RS-JIC).

In summary, here we propose to explore the genetic determinants important for the reproductive success of the emblematic Portuguese tree: *Q. suber*, that can be further used to enhance the genetic improvement of breeding stock through future markerassisted selection.