



**6 - DNA MPB\* - Title:** DNA methylation role in the periderm barrier formation

**Supervisor:** Célia Miguel, BioISI | **Co-supervisor:** Stéphane Maury, Université d'Orléans | **Co-supervisor:** Vera Inácio, BioISI

**Objectives:** Characterize the phenotypic effects of loss of DNA methylation in periderm and lenticel formation and identify epigenetically targeted genes

**Methodology:** The periderm acts as the first defense line that protects plants with secondary growth from external stresses. It is mostly composed of suberized cork cells, resulting from the activity of the phellogen or cork cambium. A high number of cork cell layers is produced by cork oak's (*Quercus suber*) phellogen which is currently the exclusive commercial source of cork used for important industrial applications worldwide. Cork cells are crossed at certain points by lenticular channels - cork pores - formed by the lenticular phellogen, that at high levels strongly depreciate cork economic value. Although the factors underlying cork quality traits are unknown, our previous results point to an association between DNA methylation and cork contrasting phenotypes directly linked to phellogen activity.

This PhD will be integrated in the EpiCORK FCT project PTDC/ASP-SIL/1717/2020 that builds on the hypothesis that key genes controlling phellogen activity and lenticel formation are epigenetically regulated via DNA methylation. A combined strategy of reverse genetics, epigenomics, chemical and transcriptomic approaches will be used to test the hypothesis in the potato periderm model. The research plan will include (I) generation of loss-of-function DNA methyltransferase mutants by an expression-inducible CRISPR-Cas9 system (at BioISI); (II) phenotypic characterization of the mutants using chemical (suberin quantification and composition), and confocal fluorescence and electron microscopy analyses (at BioISI and ISA); (III) identification of candidate genes under epigenetic regulation by correlating phellogen-specific DNA methylation and transcriptomic analyses (at BioISI and Univ. d'Orléans); (IV) assessment of chromatin accessibility and DNA de/methylation in homologs of previously identified genes and suberin biosynthesis genes in cork oak (Univ. d'Orléans). The fellow will develop the work between the Forgen lab (Ciências/BioISI, Lisboa), Instituto Superior de Agronomia (ISA collaboration, Lisboa) and Stéphane Maury's lab (Univ. d'Orléans). The supervision team will provide the expertise in genetic transformation, and transcriptomics using high throughput approaches (Célia Miguel), in tree epigenetics and whole-genome DNA methylation analyses techniques (Stéphane Maury), and in periderm anatomy and epigenetics, confocal and electron microscopy (Vera Inácio). A better understanding of the epigenetic mechanisms underlying a crucial developmental process to plant survival, particularly under a climate change scenario, and the first steps towards transferring this knowledge to the national tree, cork oak, will be achieved.

\* Mixed Fellowship (sponsors max 2yrs at an international institution through salary increase)