



Altering DNA methylation in cork oak to study its effect on cork formation

Place of work: *ForGen Lab – Forest Genomics and Molecular Genetics Laboratory* - Faculdade de Ciências da Universidade de Lisboa (FCUL) and *Genetics Lab* (LEAF, ISA)

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Abstract / MSc thesis project proposal

The periderm is a protective layer that defends plants with secondary growth from adverse environmental conditions. It is made up of cork cells produced by the phellogen and their differentiation involves chromatin condensation and de novo DNA methylation in cork oak. Moreover, DNA methylation is thought to influence the variability of cork oak periderm phenotypes directly linked to phellogen activity. Cork oak periderm is also a unique forest product – the cork, constituting the basis of a sustainable and profitable industry.

To test the hypothesis of a causal relationship between DNA methylation and the differential phellogen activity, this plan will include:

- (I) induction of DNA demethylation in cork oak seedlings and in 1- to 3-year-old plants using different DNA methylation inhibitors such as 5-azacytidine and zebularine, cytidine analogs incorporated into DNA during replication, and RG108, a non-analog of cytosine, that block the catalytic pocket of free DNA methyltransferase directly;
- (II) quantification of global DNA methylation through an ELISA-based methylated DNA quantification commercial kit in periderm tissues of treated and non-treated control plants;
- (III) analysis of the DNA methylation level of genes and their regulatory regions involved in periderm development in treated and non-treated control plants through bisulfite sequencing;
- (IV) gene expression analysis of those genes by qRT-PCR and correlation analysis with the DNA methylation levels previously assessed;
- (V) phenotypic analysis of periderm and lenticel formation through histological analysis.

Correlation of methylation levels with gene expression levels will allow identifying sites presumably involved in the control of gene expression by DNA methylation, that can be associated with the altered cork oak periderm phenotypes.

The work will be carried out within the scope of an FCT project, and the student will be integrated into a multidisciplinary team of researchers from different Portuguese institutions (ISA and FCUL). The student will acquire competencies in molecular biology, using different techniques, namely nucleic acids extraction, ELISA, PCR, qRT-PCR; histology and bioinformatics, transversal to several research areas.