



BioISI - Biosystems & Integrative Sciences Institute

Analysis of DNA methylation role in periderm formation using fluorescence activated sorted cells of potato

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

Supervisors: Vera Inácio (vlinacio@fc.ul.pt), Célia Miguel (cmmiguel@fc.ul.pt) FCUL)

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, whose differentiation involves chromatin condensation and *de novo* DNA methylation in cork oak. Also, 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.

The aim of this proposal is to test if there is a causal relationship between DNA methylation and periderm phenotype variability. To address this, we will use potato tuber periderm as a model system, which is widely used to study periderm development, by taking advantage of DNA methyltransferase silencing mutant potato plants.

The plan includes:

- (I) protoplast isolation from potato microtubers of mutant and wild type (wt) control plants;
- (II) selection of phellogen cell population by fluorescence-activated cell sorting (IGC service) from microtubers protoplasts (both wt and mutant lines harbor a fluorescence reporter gene);
- (III) analysis of DNA methylation changes in regulatory regions of genes involved in periderm formation (from phellogen induction to cell wall suberization) in phellogen sorted cells from both wt and mutant through bisulfite sequencing;
- (IV) comparison of gene expression level of the same genes through qRT-PCR in phellogen sorted cells from wt *versus* mutant and correlation with methylation levels.

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 potato periderm phenotype.

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 (nuclei acids extraction, PCR, qRT-PCR), flow cytometry, and bioinformatics, transversal to several research areas.

Approx. Duration: 6-9 months
No. of students for the project: 1