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Validation of SNP genotyping approaches for the identification of *Pinus pinaster* plants more resistant to the pine wilt disease

Place of work/: ForGen Lab – Forest Genomics and Molecular Genetics Laboratory - Faculdade de Ciências da Universidade de Lisboa (FCUL)/ Instituto de Tecnologia Química e Biológica António Xavier (ITQB-NOVA)

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The student will be part of a project that aims at confirming the association of previously identified single nucleotide polymorphisms (SNPs) in maritime pine (*Pinus pinaster*) with the species ability to survive inoculation by the pinewood nematode, the causal agent of the pine wilt disease (PWD). PWD is one of the most serious diseases currently affecting coniferous forests in East Asia and the Iberian Peninsula. It has caused high economic losses and threatened forest ecosystems in affected areas. Maritime pine showed extreme susceptibility to the disease. In recently performed work in our lab it possible to identify candidate genes involved in resistance (Modesto et al. 2021, <https://doi.org/10.3389/fpls.2021.690857>), as well as genetic variants or SNPs (Single Nucleotide Polymorphisms) possibly associated to the plant response.

The main objective of this project is to validate the usefulness of previously identified SNPs in the discrimination of PWD resistant and susceptible plants of maritime pine at an early stage of the plant life cycle. The work plan includes:

- (I) PWN inoculation trial and symptom recording
- (II) DNA extraction from tissue samples of resistant and susceptible plants
- (III) Design of primers for amplifying regions containing the selected SNPs
- (IV) SNP genotyping of DNA samples from resistant and susceptible plants by PCR and Sanger sequencing
- (IV) Analysis of the suitability of the tested SNP in plant screening

This work will be integrated in a large national project involving public and private entities aiming at the conservation and improvement of forest genetic resources in Portugal.