

**Propostas de Planos de Tese de Mestrado
Instituto de Tecnologia Química e Biológica
Genomics of Plant Stress Laboratory (GPlantS lab)**



TEMA I

Title: Analysis of OsRMC influence on gene expression changes and root mechanosensing in *Arabidopsis* under jasmonic-acid (JA)

State-of-the-art:

Phytohormones play an important role in the balance between growth regulation and tolerance/resistance mechanisms. Among plant hormones, jasmonates (JA) are one of the best characterized and studied. The signalling pathway of JA is involved in the modulation of either biotic [1] or abiotic stress [2] although most of the reports implicate this hormone in the organization of the plant defence mechanism. Nevertheless, the signalling pathway of JA has been well characterized in the recent years especially in dicots like *Arabidopsis* and tomato [3-6] but not in monocots and in particular rice.

Background:

In our lab we have been interested to understand how a rice gene, *OsRMC* (*ROOT MEANDER CURLING*) [7, 8], may be involved in the regulation of the JA-signalling pathway in rice. This gene protein appears to be modulated in rice through the action of the proteasome. The *OsRMC* seems to be involved in the modulation of abiotic and biotic stress, and root mechanosensing in rice through a negative regulation of the JA-signalling.

Aims:

With this Master thesis proposal we aim to understand how *OsRMC* may influence the JA-signalling in *Arabidopsis* and how it can affect root growth parameters and mechanosensing. We will use the *Arabidopsis* model because there is not a known homologue of *OsRMC* which makes this system useful to study its function. The MSc student will prepare genetic constructs that will be used to transform *Arabidopsis* plants and protoplasts. The MSc student will monitor gene expression changes of JA-responsive genes in the transgenic and wild-type (WT) protoplasts to assess the putative negative regulation of *OsRMC* under JA. The Master student will also measure root growth parameters and barrier response in the transgenic *Arabidopsis* plants eventually using live imaging techniques.

Place: Genomics of Plant Stress lab, Instituto de Tecnologia Química e Biológica (ITQB), Oeiras, Portugal.

Duration: 9 months

Number of students: 1

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Tasks:

1. Preparation of genetic constructs using the GATEWAY system to overexpress *OsRMC* in *Arabidopsis* plants and protoplasts.
2. Establishment of a stable transgenic protoplasts line overexpressing *OsRMC*. Analysis of gene expression changes of JA-responsive genes under JA in the transgenic line and wild-type (WT).

3. Transformation of *Arabidopsis* plants and selection of homozygous plants. Analysis of root growth rate in response to a barrier under JA of transgenic and WT plants.

Techniques:

- Preparation of genetic constructs using GATEWAY system.
- Transformation of *Arabidopsis* plants and protoplasts.
- *Arabidopsis* tissue culture.
- Total RNA extraction.
- cDNA synthesis.
- Reverse Transcriptase-PCR and agarose-gel analysis.
- Total protein extraction and quantification (Bradford).
- Total protein fractioning by SDS-PAGE and protein detection by Western blot.
- Microscopy.
- Analysis of *Arabidopsis* root growth parameters.

Bibliography:

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2. Sugano, S., et al., *Stress-responsive zinc finger gene ZPT2-3 plays a role in drought tolerance in petunia*. Plant J, 2003. 36(6): p. 830-41.
3. Chini, A., M. Boter, and R. Solano, *Plant oxylipins: COI1/JAZs/MYC2 as the core jasmonic acid-signalling module*. Febs J, 2009. 276(17): p. 4682-92.
4. Staswick, P.E., *JAZing up jasmonate signaling*. Trends Plant Sci, 2008. 13(2): p. 66-71.
5. Thines, B., et al., *JAZ repressor proteins are targets of the SCF(COI1) complex during jasmonate signalling*. Nature, 2007. 448(7154): p. 661-5.
6. Chini, A., et al., *The JAZ family of repressors is the missing link in jasmonate signalling*. Nature, 2007. 448(7154): p. 666-71.
7. Jiang, J., et al., *RNAi knockdown of Oryza sativa root meander curling gene led to altered root development and coiling which were mediated by jasmonic acid signalling in rice*. Plant Cell Environ, 2007. 30(6): p. 690-9.
8. Zhang, L., et al., *Identification of an apoplastic protein involved in the initial phase of salt stress response in rice root by two-dimensional electrophoresis*. Plant Physiol, 2009. 149(2): p. 916-28.