

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



Title: Unveiling SUMO conjugating enzymes role in rice (*Oryza sativa*)

Rice (*Oryza sativa*) is one of the most important food crop plants in the world, but it is also particularly sensitive to abiotic stress conditions, mainly drought, cold and salinity. With the goal of finding and characterizing the different tolerance strategies we are studying SUMO conjugating enzymes (SCE1), which are the enzymes responsible for catalyzing the bond between SUMO and the target protein, in a process known as sumoylation. Sumoylation is a post-translational modification which has been shown to be involved in abiotic stress response [1]. Moreover, mutation of some of the components of the Arabidopsis sumoylation machinery (mainly the only SCE1 gene) leads to embryo lethality. Mutant lines of each of the three OsSCE1 genes in rice show a dwarf phenotype and higher infertility, thus showing the putative agronomic interest of these genes. In this project we hope to understand the role of each OsSCE1 gene in rice and address their importance in abiotic stress response.

Background and aims:

In our lab we have been studying rice sumoylation machinery, and we have acquired/developed mutant lines of the three OsSCE1 genes (*Ossce1a*, *Ossce1b* and *Ossce1c*). Their promising phenotype together with preliminary results showing their different response in drought stress, led us to propose that these genes are not redundant and might have different functions/targets. In order to determine the most relevant gene and isoform, since each gene has two alternative splicing isoforms, the master student will address the different OsSCE1 transcript profiles in the mutant lines and also if the mutant sumoylation levels are affected. These tasks will be performed under control and drought stress conditions. After unraveling which isoforms are the most important/responsive, the student will over-express it in Arabidopsis, obtain homozygous transgenic lines and characterize them at the molecular and phenotypic level, in both control and stress conditions. This study will provide new insights regarding rice SCE1s function and ultimately contribute to understand plant stress tolerance mechanisms.

Tasks:

1. Analysis of OsSCE1 isoforms expression in the mutant lines (*Ossce1a/b/c*) and in wild type (WT), under control and drought stress conditions.
2. Analysis of sumoylation levels in the mutant lines.
3. Preparation of genetic constructs using the GATEWAY system to over-express the candidate isoform in Arabidopsis plants.
4. Transformation of Arabidopsis plants with *Agrobacterium* and selection of homozygous plants.

5. Phenotypic and molecular characterization of the transgenic lines obtained and wild type under control and drought stress conditions.

Techniques:

- Total RNA extraction.
- cDNA synthesis.
- Reverse Transcription-PCR and agarose-gel analysis.
- Western blot.
- Preparation of genetic constructs using GATEWAY system.
- Transformation of Arabidopsis plants with *Agrobacterium tumefaciens*.
- Characterization of Arabidopsis molecular-phenotypic parameters.

Place: Genomics of Plant Stress Unit, Instituto de Tecnologia Química e Biológica (ITQB), Oeiras, Portugal, under direct supervision of Dr. Isabel Abreu and Prof. Margarida Oliveira and working together with PhD Student Margarida Rosa.

Duration: 9 months

Number of students: 1

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Bibliography:

1. Kurepa, J., et al., *The small ubiquitin-like modifier (SUMO) protein modification system in Arabidopsis. Accumulation of SUMO1 and -2 conjugates is increased by stress.* J Biol Chem, 2003. **278**(9): p. 6862-72.