Stage M2 2020-2021

Tuteur du stage et Laboratoire d'accueil :
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Research project title:
Plant speed-dating: Deciphering the early molecular dialog between reproductive partners

Project description:
Plant reproduction depends on a random rendez-vous, the first contact being when hundreds of pollen grains, carrying the male gametes, land on the surface of the female reproductive organ (the stigma). In flowering plants, sophisticated mechanisms allow the stigma to reject genetically-related (self or incompatible) pollen while accepting non-self (or compatible) pollen. These self/non-self-recognition mechanisms, known as Self-Incompatibility (SI), prevent self-fertilization and promote genetic variability within the species. In the Brassicaceae family including the model plant *Arabidopsis thaliana*, SI is controlled by a receptor-ligand interaction at the stigmatic surface, which involves the stigmatic SRK receptor (S-locus Receptor Kinase) and its pollen specific ligand SCR (S-locus Cysteine-Rich) (Ivanov et al., 2010; Figure 1).

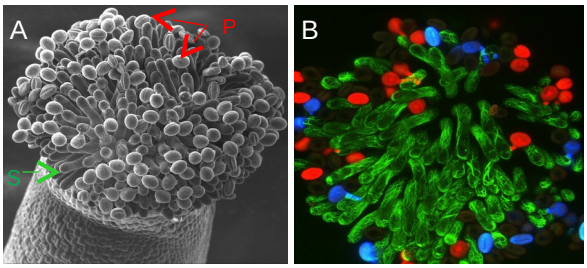

Figure 1: Cell-cell interaction at the female organ surface. (A) Scanning Electron Microscopy of a pollinated stigma. (B) Stigmatic cell expressing a cytoskeleton marker (green) pollinated with compatible pollen grains expressing a cytoplasmic red or blue marker (confocal microscopy).
S: stigmatic cells; P: pollen grains

The research project aims at understanding the downstream signaling cascade that occurs at the female organ surface following pollen perception. We recently carried out a transcriptomic analysis and identified two Receptor-Like Kinases (CRK31 and CRK41) highly upregulated in the stigma upon incompatible pollen recognition. These two receptors have well described functions in the regulation of defense reactions against pathogens thought Rapid Oxygen Species (ROS) signaling but up to now, their roles in reproduction have never been described.
In this internship project, we propose experimental approaches to decipher the role of CRK31/41 together with ROS-signaling in incompatible pollen rejection in *A. thaliana*. First, the master student will generate transgenic lines in which expression of CRK31 and/or CRK41 are invalidated using the CRISPR/Cas9 technology and will determine whether the pollination phenotype is altered using fluorescence as well as Scanning Electron Microscopies. Second, the anticipated physical interaction between SRK and CRK31/41 will be examined by bimolecular fluorescence complementation (BiFC) and immunoprecipitation experiments. Third, using a tool to directly monitor dynamic redox changes in living cells, the student will generate transgenic plants to test, by confocal microscopy, if pollination modifies the redox state of stigmatic cells. In addition, to test the possible implication of ROS in SI signaling, mutants impaired in ROS production will be identified and analyzed.

Some publications from the group:
Rozier et al. (2020) *J Exp Bot* 71, 2513–2526. Riglet et al., (2020) *eLife* 9:e57282. Burghgraeve et al., (2020) *Genetics* 215, 653-664. Durand et al. (2014) *Science* 346, 1200-05. Ivanov et al. (2010) *Trends in Plant Science* 15: 387-394.