MASTERBIOSCIENCES
ECOLE NORMALE SUPERIEURE DE LYON

Offre de stage de Master / Master Internship offer

Tuteur du stage et Laboratoire d'accueil / Internship supervisor and Host laboratory:
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Titre du projet de recherche / Research project title:
Plant speed-dating: Deciphering the first molecular dialogue between reproductive partners

Description du projet / 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, 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).

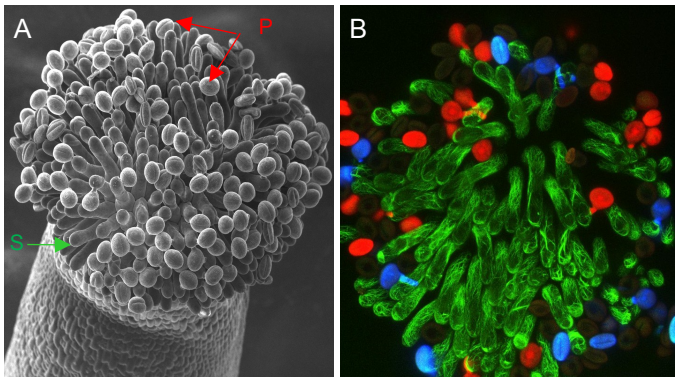

Figure 1: Cell-cell interaction at the female organ surface. (A) Scanning Electron Microscopy of a pollinated stigma. (B) Stigmatic cell expressing a cytoskeleton marker (green) pollinated with compatible pollen grains expressing a cytoplasmic red or blue marker (confocal microscopy).
S: stigmatic cells;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

The research project aims at unraveling the downstream signaling cascade that occurs at the female organ surface following pollen perception. We recently carried out a transcriptomic analysis and obtained a catalog of genes whose expression is rapidly modified after compatible or incompatible pollen-stigma interactions. From this list of differentially expressed genes (DEGs), we selected candidate genes that presumably have function for long-term maintenance of SI. Our top candidates are two Receptor-Like Kinases belonging to the same family (RLK1 and RLK2). We are currently producing transgenic *Arabidopsis* lines in which expression of RLK1 and/or RLK2 is invalidated using the CRISPR/Cas9 technology.

The proposed internship research project consists in a thorough analysis of these CRISPR lines by using state-of-the-art techniques. First, the master student will determine whether the pollination phenotype of these plants is altered using fluorescence as well as Scanning Electron Microscopy. Second, she/he will be involved in the analysis of the subcellular localization of these two RLKs. For that purpose, transgenic plant expressing GFP-RLK fusion protein will be generated and analyzed by confocal microscopy. Third, the anticipated physical interaction between SRK and these two RLKs will be examined by bimolecular fluorescence complementation (BiFC) and immunoprecipitation experiments.

Publications du laboratoire (5 max) / Lab publications (5 max):
Ivanov *et al.* (2010) Trends in Plant Science 15: 387-394. Durand *et al*. (2014) *Science* 346, 1200-1205.