TULIP LabEx

Application form to the "Young Scientists for the Future" Call 2016

- **Research Unit of the PhD student:** **LIPM**

- **Thesis supervisor:** **Dominique ROBY** **Co-Supervisor:** **Fabrice ROUX**

- **Number of post-docs and PhDs currently supervised :** **Dominique Roby: 1post-doc, 0.5 PhD - Fabrice Roux : 2 x 0.5 PhD**

- **Thesis project title:** **Plant response to multiple pathogen strain infection: An ecological genomics approach for the dissection of underlying molecular mechanisms**

- **Thesis project description (1.5 page maximum) (Scientific context, Previous results, Project description, References)**

Scientific context. Pathogen species are a threat for crops and natural plant populations. There is thus an acute obligation to better understand the mechanisms underlying pathogen aggressiveness and plant disease resistance or susceptibility and to find new, durable and sustainable means to combat crop diseases. The importance and complexity of pathogen perception and signaling pathways in the regulation and execution of plant immune responses have become apparent during the last years. Notably, *R* gene-mediated immunity has been shown to be the most efficient form of resistance in plants, but it is also costly and not durable. Additional forms of resistance have gained increasing attention for breeding purposes, such as quantitative disease resistance (QDR), but they are still poorly understood. QDR is much more prevalent than specific resistance in crops and natural plant populations (1), and is a form of resistance which leads to a reduction in disease, rather than an absence of disease (2). The molecular architecture of QDR can be hypothesized as an intricate network integrating multiple response pathways to several pathogen molecular determinants and environmental cues (3). In addition, the knowledge gained in recent years on the molecular determinants of qualitative resistance (ETI, PTI) only represents the tip of the iceberg, whereas QDR-specific molecular components and mechanisms, and their connections to other signaling pathways, are still to be discovered.

Xanthomonas species are a major constraint for seed and plant production in vegetables crops, especially in Brassica (4) and Solanaceae, causing black rot and bacterial spot, respectively. Plant pathogenic *Xanthomonas* species have a wide geographical distribution, causing reduction in yield and quality and are expected to increase in incidence and range under climate change (5). We recently identified (6) *RKS1* as a quantitative resistance gene in *Arabidopsis thaliana* conferring broad-spectrum resistance to *Xanthomonas campestris* (Xc). Interestingly, *RKS1* encodes an atypical kinase. Its functional analysis, together with the dissection of the resistance pathways activated downstream, is under study in our lab. We demonstrated that RKS1-dependent immunity is effective not only against the strain Xcc568 of Xcc (race 3), but also against strains in four additional races of Xcc (races 1, 5, 7 and 9). However, RKS1 does not confer resistance to all races of Xcc, and we identified a gene of unknown function conferring QDR to Xcc12824 (race 2), and the dual resistance gene system *RRS1/RPS4* as being involved in QDR to XccCFBP6943 (race 6), suggesting that QDR to Xc involves a complex network integrating multiple response pathways triggered by distinct pathogen molecular determinants (7). While of major interest, those findings are based on experiments in which plants have been challenged with only one strain at a time.

However, in natural populations, plants generally interact simultaneously with several strains of the same pathogen species, leading to the challenges of linking functional genomics and community ecology (8). The identification of the genetic determinants for this "multi-strain" response represents a major challenge to

understand the pathways that ultimately enable a plant to fine-tune its defense against different aggressors. In addition, this would undoubtedly shed some light on downstream components of the complex signaling network leading to resistance and that cannot be revealed by classical (mono-strain) approaches.

Previous results. In a preliminary experiment, 48 accessions of *A. thaliana* have been either inoculated with strains Xcc568 (race 3) and XccCFBP6943 (race 6) once at a time, or co-inoculated with both strains at the same time. We observed genetic variation for the response to co-infection. More importantly, the response to multi-strain inoculation cannot be described as an additive function of response to single-strain inoculation, suggesting that the genetic bases associated to co-inoculation infection differs from the genetic bases associated with mono-inoculation. Accordingly, based on 214k SNPs, we adopted a Genome Wide Association mapping approach and identified QTLs that are specific to co-inoculation (i.e. that are different from *RKS1* and *RRS1/RPS4*).

Project description. The objectives of the project will be to elucidate the genetic and molecular bases of the plant response to different strains of the bacterial pathogen *Xanthomonas campestris*, and will include four main steps:

(i) To identify the genetic bases of the multi-strain response by a Genome Wide Association mapping approach, a set of 200 accessions of whole genome - sequenced *A. thaliana* will be phenotyped for quantitative resistance in the context of single-strain inoculation and multi-strain co-inoculation.

(ii) To functionally validate the gene(s) underlying the QTL(s) conferring quantitative resistance to multi-strain co-inoculation. This will be performed by phenotyping of (i) mutants affected in this/these candidate gene(s), (ii) complemented mutant lines, and (iii) candidate gene overexpressing lines. This last point will be envisaged according to the candidate gene function.

(iii) To integrate this/these genes within the RKS1 dependent QDR pathways that is currently dissected through different complementary approaches and more widely in the existing defense pathways known in plants. This part of the project will show whether QDR resistance pathway(s) operate(s) independently or through common regulatory nodes with other forms of resistance.

(iv) To study the selective forces acting on the genes functionally validated.

Given the paucity of quantitative resistance genes identified in plants, this project will be a major break-through in the understanding of the molecular mechanisms underlying quantitative resistance and a first step toward the understanding of the plant response to multiple pathogens.

References.

(1)Young, 1996, *Annu. Rev. Phytopathol.* 34, 479-501.; (2) Poland *et al.,* 2009, *Trends Plant Sci.*14: 21-29.; (3) Roux *et al.,* 2014, *Molecular Plant Pathology* 15(5), 427-432. doi: 10.1111/mpp.12138; (4) Williams, 1980, *Plant Disease* 64: 736 -742. ; (5) Boland *et al.* 2004, *Canadian Journal of Plant Pathology* 26: 335-350.; (6) Huard-Chauveau *et al.,* 2013, *PLoS Genetics* 9: e1003766. ; (7) Debieu et al., 2015, *Mol. Plant Pathol.* 17(4):510-20. doi: 10.1111/mpp.12298 ; (8) Roux & Bergelson, 2016, Curr. Topics dev. Biol.

- **Project summary (200 words) :**

In nature, plants are constantly submitted to attack by multiple bioagressors and have evolved a complex multilayered immunity system. One component of the immune system is the Quantitative disease resistance (QDR). This is a form of resistance which leads to a reduction in disease, rather than an absence of disease and is highly prevalent in crops and natural plant populations. We recently identified *RKS1* (encoding an atypical kinase) as a quantitative resistance gene in *Arabidopsis thaliana* conferring broad-spectrum resistance to *Xanthomonas campestris* (Xc) and other QDR genes more strain-specific. However, in natural populations, plants generally interact simultaneously with several strains of the same pathogen

species, and a major challenge in plant pathology is now to understand how a plant fine-tune its defense against different aggressors. By combining cutting edge expertise in genetics, plant molecular and cell biology, and evolutionary ecology, the objective of this project is to identify the genetic and molecular bases of such a multi-strain response, and to integrate these new signaling components within the RKS1 dependent QDR pathways currently under dissection. Such identification represents a unique opportunity to compare the selective forces acting on genes associated to different levels of complexity of biotic interactions.