

PROJETS DEFIS SCIENTIFIQUES 2018

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CliSeTiCS

Project title: Quantifying and modelling Climatic Services provided by Trees inside a Canyon Street

Acronym: CliSeTiCS

Project duration: 36 months – Start date: 01/10/2018 End date: 30/09/2021

Key-words: Water, soil, plant, agronomy, urban, ecosystem services, heat island, modelling, transpiration

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Financial support from « Objectif Végétal »: 25k€

Summary:

Context

More than half of the world population lives in cities, thus maintaining well-being in cities appears as a critical point. Plants are a key element in urban planning, given the ecosystem services they provide, especially the role they play in heat island mitigation and refreshment. Within the next decades, however, with the climate change and global warming, extreme events will occur more frequently (heat waves, drought). In this context, the competition for water usage will increase, and keeping plants in a good ecophysiological state will thus become a challenge not only to warranty their sustainability, but also to maintain their ecosystem services cited above. Given this context, stakeholders request tools adapted to the specificity of urban conditions in order to plan greening for towns of tomorrow.

Goals

The project aims at better understanding and quantify the climatic ecosystem services provided by trees in cities in terms of refreshment and shading under different climatic conditions and at evaluating the consequences of water restriction on these services. For the purpose of the project, trees grown inside a canyon street will be considered. Indeed, the canyon street infrastructure is a typical urban configuration in many towns and the simplicity of its geometry will facilitate the modelling stages. The PhD thesis will focus on the quantification of water transfers through the substrate-plant-atmosphere continuum under urban conditions, and on the heat transfers between the trees and their environment for different water regimes.

Methodology

To reach this goal the project will include:

- (i) a characterization of the physical processes involved in water and energy transfers through the soil-plant-atmosphere continuum on the basis of a state-of-the-art review, and from an analysis of data recorded in urban conditions,
- (ii) the quantification of these processes from experiments conducted on a reduced-scale model (canyon street scale 1:5) with well-watered trees and/or trees under water restriction. The question of how these results can be transposed to the real urban scale will be analyzed at that stage,
- (iii) a modelling of the dynamics of those transfers and of their consequence on the climatic ecosystem services provided by trees when grown under water comfort or water restriction conditions. A global model on the one hand and a distributed climate model on the other hand (based on a Computational Fluid Dynamics-CFD tool) will be used, a validation against measured data will be performed.
- (iv) the simulation of different climates and water inputs scenarios in order to assess the ability of trees to provide the expected climatic ecosystem services for a range of climatic and environmental conditions.

FuSloN

Project title: Role of nitrogen in plant-fungal pathogen interactions during seedling establishment

Acronym: FuSloN (Fungus Seedling Interaction Nitrogen)

Project duration: 36 months – Start date: 01/10/2018 End date: 30/09/2021

Key-words: *Arabidopsis thaliana*, *Alternaria brassicicola*, Sustainable agriculture, Defence responses, Nitrate transporters, Nitrogen metabolism, Nutrition, Signalling.

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Summary:

Context

Successful crop establishment relies on the ability of the seeds to germinate and establish seedling rapidly and uniformly. However, early stages of plant development are highly susceptible to environmental variations. With the development of sustainable agriculture, limitation of chemical uses for plant fertilization (nitrate supply) also concerns these early stages. In addition, rules and laws regarding seed treatments will dramatically evolve in the next future leading to reduction of seed treatments for example with fungicides. In this context, the control of seed-borne fungal pathogens present on seeds or in the soil will be less efficient, increasing the risk for seedlings to be exposed to disease development. Nitrogen (N) is a metabolic resource at a central place of plant-fungal pathogen interactions. On one side, pathogens require N for their development and infection process. On another side, seedling development also requires N, which can be provided from seed reserve mobilization, but also from nitrate, a naturally occurring form of N in the soil and a nutrient known to regulate seedling growth as a signal molecule. The levels of nitrate supply may also dramatically affect the interplay between host and pathogen for N acquisition. In this context, the role of N in the plant-fungal pathogen interactions during seedling establishment taking into account various nitrate supply levels requires investigation.

Goals

This project, which gathers expertise of two teams of UMR IRHS "Seedling, Metabolism and Stress" (SMS) and "Pathologies fongiques des semences" (FungiSem), aims at deciphering the role of nitrogen in plant-fungal pathogen interactions during seedling establishment, using the pathosystem *Arabidopsis thaliana* - *Alternaria brassicicola*, a seed-borne necrotrophic pathogen causing damping-off disease of seedlings leading to important economic losses.

Methodology

Plant stages from seed germination to seedlings with four rosette leaves will be considered. Experiments will be carried out at two contrasting nitrate levels, corresponding to the natural concentration in a non-fertilized soil and to a non-limiting one, and taking advantage of comparison of the wild-type Col-0 *Arabidopsis* genotype with mutants for the gene *AtNPF6.3* encoding a nitrate transporter. Following this strategy; the importance of nitrate supply for the susceptibility of *Arabidopsis* to *A. brassicicola* will be assessed during seed germination and seedling development as a function of nitrate supply conditions. Metabolite profiling i.e. contents of nitrate and amino acids, hormones involved in plant defence signalling pathways, defence metabolites, and expression of plant and fungal genes potentially involved in N metabolism and defence during the plant-pathogen molecular interactions will be analysed during seedling development after seed inoculation with *A. brassicicola*.

GOSYRIS

Project title: Garden Orchard SYstems: Research Into the Soil-Plant-Atmosphere Continuum

Acronym: GOSYRIS

Project duration: 36 months - Start date: 01.11.2018 End date: 31.10.2021

Key-words: soil-plant-atmosphere continuum (SPAC); garden orchard; agroforestry; water and nitrogen balance

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Financial support from « Objectif Végétal »: 15 k€

Summary:

Context:

Driven by societal demands, the design of Territorial Food Systems leads to the development of innovative fruit and vegetable production systems in periurban zones. These production systems have to accommodate multiple objectives: (1) produce in sufficient quantity and quality, (2) optimize pesticide use and minimizing waste (with respect to human health and protection of neighbouring population), and (3) limit waste of resources. The concept of Agroforestry appears to be a suitable answer to this double challenge, namely of ecological intensification of production, and autarky of field-cropping systems. In Third World countries (countries of the Global South), the introduction of shade trees in field crop culture has been studied from an agronomic and socio-economic perspective, and the results of these studies are very promising. Closer to home, agroforestry systems consisting of timber trees and cereals have also been studied (Dufour et al., 2013). However, their application to a specific agroforestry system, the "garden orchard" is not a straight affair, for several reasons: (1) there is a high diversity and a rapid succession of vegetable rotations which renders the search for a suitable supplies and requirements for water and minerals rather complex, and (2) numerous agronomic interventions conducted on the fruit trees potentially induce a significant variability in the functioning of tree roots.

Goals:

The objective of the project is to produce a model of the functional traits describing the soil-plant-atmosphere continuum system in a garden orchard. Based on the identification and hierarchisation of the different processes that are intervening in the regulation of water and mineral uptake and transport, this conceptual model will be formalized mathematically and then algorithmically implemented as a computer simulation, before being validated, both in an experimental and a professional context. As an immediate perspective, it will allow the formalisation of decision-support tools (DST) for better management of garden orchards.

Methodology:

To reach this goal, the project will include

1. a characterization of the physical and chemical processes interacting through the soil-plant-atmosphere continuum (SPAC) based on a state-of-the-art review,
2. a quantification of these processes thanks to experimental field surveys on two garden orchards including microclimate data, physical and chemical properties of the soil, as well as plant growth and yield, with a spatial and temporal resolution sufficiently detailed to allow the observation of the effect of the trees,
3. the creation or adaptation of a model of the garden orchard in order to establish the efficiency of the system, in terms of yield, carbon and nitrogen balance, depending on its management,
4. the simulation of different management scenarios to validate the model, along with a catalogue of proposed measures to improve and adapt the studied system.

IMAGINES

Project title: Towards the production of pea ideotypes more efficient in soil nitrogen absorption for improving weed control in low input innovative intercrops

Acronym: IMAGINES

Project duration: 36 months - Start date: 1 October 2018 End date: 30 September 2021

Key-words: Intercropping, legume, weed contrai, nitrate absorption, biological nitrogen fixation, transceivers, lateral roots, Nod factors

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Financial support from « Objectif Végétal »: 25 k€

Summary:

Context

In a context of increasing protein demand and limitation of nitrogen fertilizers and pesticides use, intercrops including a legume providing one or more services, are seen as a means to move towards productive systems more sustainable and resilient to climatic and economic hazards. Recent work has shown that it is possible to use auxiliary legumes for limiting weed development and providing a cash crop such rapeseed with atmospheric nitrogen resulting from biological fixation. The productivity of this kind of intercrops strongly depends on competition and complementarity for the use of light and nitrogen between associated crops and weeds. Indeed, for controlling weeds, it is necessary that the rapeseed and the legume effectively absorb the soil nitrogen at the expense of the weeds. However, legumes are low competitors for soil nitrogen, likely because they also can fix atmospheric nitrogen through root nodules resulting from symbiosis with bacteria

Objectives

The objectives of the project IMAGINES is to better understand interactions between the development of lateral root for a higher absorption of soil nitrogen, soil nitrate concentration and the presence of bacteria allowing for nitrogen fixing nodules. It will help to design new varieties of legumes to be used as auxiliary crops, well adapted to their expected functions for improving the performance of intercrops.

Methodologies

Pea plants will be grown in greenhouse to study the effect of soil nitrate concentration on lateral root development and root architecture. The effect of the presence of nitrogen-fixing bacteria on root architecture will then be studied as well as the interaction with the mineral nitrogen uptake of the soil. The pea roots will also be phenotyped in the presence of rapeseed and weeds. Finally, the interactions between the elongation of lateral roots, soil nitrate and fixing bacteria will be studied by molecular techniques.

PROMETEUS

Project title: PRi Optimizatinon by dna MEthylation and Transposable Elements studies: Unraveling their persistence and performance modulation by environmental constraints; an apple case Study

Acronym: PROMETEUS

Project duration: 36 months – Start date: 01-11-2018 End date: 31-10-2021

Key-words: Plant resistance inducers, epigenetics, transposable elements, immune memory, heat stress

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Financial support from « Objectif Végétal »: 25 k€

Summary:

Healthy apple production requires an important number of chemical pesticide treatments. Our team showed that repeated PRI (Plant Resistance Inducer) applications significantly reduces the number of pesticide treatments required to protect the trees against apple scab, a fungal disease responsible for the half of the treatment frequency index of apple orchards. In order to optimize this new protection method, we propose to deepen the knowledge on the mode of action of two PRI by studying two aspects: (i) plant ability to memorize PRI treatments and (ii) plant ability to respond more or less efficiently to PRI applications when facing environmental constraints. First of all, the effect of two PRIs will be monitored by DNA methylation assays, transposable elements (TE) activation and gene expression analysis at the genome scale. Regarding memorization, the hired PhD will study by focused analyses the maintenance/inheritance of DNA methylation at different timescales: week, months and from one generation to the other. Regarding environmental constraints, the PhD project will focus on the effect of high temperatures on apple PRI response ability. Global analyses of gene expression, DNA methylation and TE activation of PRI-treated apple plants facing heat stress will therefore be performed and compared to those obtained with either PRI-only treated plants, either heat-only treated plants. Heat stress is the chosen environmental constraint because it (i) significantly reduces apple response to PRI and (ii) is known to activate transposable elements in other plant models. This study will allow to identify memorization and plant-receptivity processes and markers which will hopefully contribute to optimize application frequency and identify favorable conditions for successful PRI treatments.

ROSAPEPS

Project title: Rosa miPEPs and miRNAs: new friendly-environmentally tools to control branching?

Acronym: ROSAPEPS

Project duration: 36 months - Start date: 01/10/2018 End date: 30/09/2021

Key-words: Plant resistance inducers, epigenetics, transposable elements, immune memory, heat stress

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Financial support from « Objectif Végétal »: 25 k€

Summary:

Context:

In ornamental horticulture, well branched plants with many flowers are a guarantee of quality. However, the development of new branches by a plant is highly sensitive to environmental factors: adverse temperature or light conditions can induce an inhibition of buds growth causing thus the absence of new branches. This problem is critical in dense culture conditions and leads to the downgrading of the horticultural products. In gardens and especially in urban conditions, very fluctuating climatic conditions such as hot spots, prolonged shading by buildings, etc ... also negatively impact aesthetic quality of ornamental plants by a poor branch renewal. This problem is further heightened by the current climate disruption. Varieties better adapted to these stressful environmental conditions are therefore sought.

Goals:

In this context, ROSAPEPS project aims to evaluate, for the first time, the potential of microRNAs (miRNAs) and of micropeptides (miPEPs) to enhance branching of ornamental species under environmental stress conditions, low light here. miRNAs are a class of small RNAs (20-24 nucleotides) recently discovered that is largely disseminated in the genome and emerged as major regulators of plant development regulation in response to environmental factors. They act by cleaving their mRNAs target or by repressing their translation into proteins. The miPEPs are, on their side, the small regulatory peptides encoded by the primary transcripts of the miRNAs that enhance specifically their expression. To-date, very few is known on plant branching regulation by miRNA/miPEP, and even less in response to adverse light conditions. However, the use of these small regulatory molecules, either through varietal selection (for miRNAs) or by direct application on plants (for miPEPs) could offer powerful and friendly-environmentally tools to address these problems.

Methods:

The work will be conducted on rose bush, a major ornamental plant in France and particularly for production in the Pays de Loire. Rose bush will be grown under light-limiting conditions and their buds and nodes harvested to determine miRNAs involved in the control of branching in response to the light environment (thanks to rose genome sequence availability and to new high throughput miRNAs sequencing technologies). Potential target genes for these miRNAs will be identified by searching for their cleavage site along the annotated genome. The spatio-temporal expressions of some of the identified miRNAs/target gene module will be studied (in situ hybridization, qPCR) and their function validated through Arabidopsis thaliana transformation. This work will, therefore, on the one hand, contribute to better understand miRNAs mode of action in the control of branching and on the other hand, open innovative avenue for breeding based on the most relevant miRNAs/target gene module.

In addition, miPEPs will be synthesized from the miRNAs most active in branching control and will be applied to rosebush to evaluate the potential of these molecules as new friendly-environmentally tools in the control of branching in production.

ValoRGPomme

Project title: Effective use of genetic resources in the apple elite breeding population with genomic selection

Acronym: ValoRGPomme

Project duration: Project duration: 36 months – Start date: 01/11/2018 End date: 31/10/2021

Key-words: genetic diversity; introgression; breeding scheme; apple

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Financial support from « Objectif V eg etal »: 17 k e

Summary:

Context:

Apple is the second most important worldwide cultivated fruit crop and the first in France. In apple breeding programs, phenotyping for horticultural traits is lengthy and costly and marker assisted breeding, more precisely genomic selection for traits with complex genetic control, is a promising and innovative strategy. This strategy relies on the building of a genome-wide prediction (GWP) model thanks to a genotyped and phenotyped training population and the application of the model to genotyped candidates when their phenotypes are not yet available. Worldwide, elite breeding populations are most often derived from the most used cultivars and thus contain a reduced diversity. To reduce the vulnerability induced by this situation, it is necessary to enlarge the genetic diversity used in the breeding programs by an efficient use of genetic resources.

Goals:

The overall goal of the PhD project is to study how to efficiently use genomic selection to introgress favorable alleles from genetic resources into elite germplasm. More specifically, the PhD student will address the following research questions: i) how to combine data on elite and genetic resource material to apply genomic prediction to hybrids between these two gene pools? ii) What is the best genetic make-up of the training population and the best GWP model to transfer favorable alleles from genetic resources into elite germplasm? iii) would first, second or later generation hybrids between elite and genetic resource material, or hybrids from pseudo-backcross, provide more accurate GWP models?

Methodology:

Two complementary approaches will be followed to study genotype imputation accuracy and genomic prediction accuracy:

- available datasets from the apple INRA breeding program and from European genetic resources will be used with various resampling strategies and/or various statistical models
- simulations will enable to explore layouts not covered by already available data, more specifically those concerning advanced backcross or intercross generations.

CRISPR-FIRE

Project title: Durable resistance of apple against fire blight: functional validation of a major QTL through gene editing

Acronym: CRISPR-FIRE

Project duration: 18 months - Start date: 3/10/2018 End date: 2/04/2020

Key-words: apple, fire blight, resistance, durability, cloning, gene-editing, CRISPR

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Financial support from « Objectif Végétal »: 50,9 k€

Summary:

Context:

Fire blight caused by the bacterium *Erwinia amylovora* has a major economic impact because many apple and pear varieties are very susceptible to this disease. In Europe, antibiotic applications are forbidden, therefore chemical control methods are limited and the release of resistant varieties is necessary. Only one major fire blight resistance gene has been cloned in apple and it is already overcome by several strains of the bacteria. We have decided to study a resistance source a priori durable because not yet overcome in culture: 'Evereste'. This pollinator variety carries a QTL of resistance with a very strong effect, independently identified in two apple genotypes ('Evereste' and *Malus floribunda* #821). In collaboration with ETH-Zürich, the QTL region has been precisely mapped on chromosome 12 and reduced to 78 kb.

Goals:

The aim of the project is the functional validation of the fire blight resistance QTL by gene editing and the cloning of responsible gene(s). In the long term the goal will be to propose a prototype of resistant variety developed by intragenesis or by knock-in via CRISPR. Such models are necessary to initiate a debate over the advantages/drawbacks of this type of biotechnological approaches for fruit tree breeding.

Methodology:

The project is based on two innovative advances recently achieved at IRHS: i) the high quality sequencing of an apple genome ii) the mastery of a CRISPR knock-out method for this species. This last method belongs to the « New Breeding Technologies » and has never been applied for a functional validation of an apple gene.

ALDAUTOX

Project title: Biosynthesis and biological activity of aldaulactone, a fungal toxin involved in the pathogenicity of *Alternaria douci*, the main causal agent of carrot leaf blight

Acronym: Aldautox

Project duration: 24 months - Start date: 01/01/2019 End date: 31/12/2020

Key-words: polyketides, fungal toxin, plant cell cultures, yeast transformation, labelled analogues.

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Financial support from « Objectif Végétal »: 24k€

Summary:

Context

The pathogenicity of *Alternaria douci*, the main fungal necrotrophic pathogen causing leaf blight on carrot, is at least partly based on toxin production (Lecomte et al., 2014). These toxins, such as aldaulactone, a polyketide recently uncovered by Courtial et al. (submitted), are used by the fungus to kill the plant cells and grow inside the plant. Toxin biosynthetic pathways and their mode of action are currently unknown.

Goals

The aims of this project are to characterize the biosynthetic pathway of aldaulactone and to study its mode of action in the plant cell. Aldaulactone is one of the main toxin produced by *A. douci* and is, in that way, a crucial element to uncover how the pathogen attacks carrot leaves, leading to a reduction of root production or seed formation.

Methodology

Polyketide synthase (PKS) genes, selected from a transcriptomic analysis previously performed in *FungiSem*, will be used to transform the *Pichia pastoris* yeast. The toxicity of yeast extracts after transformation with PKS genes will be tested on carrot cell cultures by comparison with toxicity assays performed with pure aldaulactone. Chemical analysis of metabolites found in (or isolated from) taxie extracts produced by the transformed yeast will allow identifying and defining the synthetic pathway of aldaulactone. Toxin mode of action will be studied through a multi-step strategy. First semisynthetic modifications of the substitution pattern of aldaulactone will lead to new analogues whose toxicity on carrot cells will be evaluated. It will help us identifying both the structural moieties of aldaulactone that can be transformed without any decrease of its toxicity and the moieties that support its biological activity. Then new analogues, either bearing a fluorescent probe or a reactive linker for click chemistry reactions or exhibiting ¹³C isotopic enrichment, will be semisynthesized. These derivatives are chemical tools that could be used to track the toxin and/or its metabolites.

PAXAP

Project title: Development of tools to characterize the interaction *Xanthomonas arboricola* pv. *Pruni* / *Prunus armeniaca*

Acronym: PAXAP

Project duration: 24 months - Start date: 01/01/2019 End date: 31/12/2020

Key-words: wild compartment, centre of origin, *Xanthomonas*, apricot, detection, phenotyping

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Financial support from « Objectif Végétal »: 24 k€

Summary:

Xanthomonas arboricola pv. *pruni* (Xap) is responsible for the bacterial spot of *Prunus* spp., a disease in continuous expansion and with major economic impacts on stone and nut fruit tree production. It has been shown that the centre of origin of cultivated crops could play an important role in pathogen emergence. However, no information is available on the presence of *Xanthomonas* bacteria in the centres of origin of fruit trees. This “Starter” project aims at developing tools to explore the interaction between Xap and one of its host *Prunus armeniaca* (apricot tree). It is based on a synergistic collaboration between two INRA teams that have complementary skills and resources. In a first step, molecular detection tools will be developed thanks to genomics data of Emersys team, to explore the presence of *Xanthomonas* in lyophilized samples of wild apricot trees from Central Asia collected by partner2. Positive results would support the future setup of a sampling expedition in Central Asia to get bacterial isolates of *Xanthomonas*. In addition, protocols and detection tools setup in the frame of this project will be useful to search for the presence of Xap in herbarium. The second objective of this “starter” project is to setup a high throughput phenotypic test using detached leaves of apricot trees to assess on one hand bacterial strain aggressiveness and on the other hand host susceptibility. In future projects, this phenotypic test will allow us to compare the aggressiveness of strains isolated on natural populations in the centres of origin to the one of strains isolated in agroecosystems. These data are essential for the understanding of bacterial adaptation to their host. The phenotypic test will also allow to assess the susceptibility to Xap of wild apricot trees from Partner2 collection. The genome of 80 wild apricot trees has been sequenced; the implementation of this phenotypic test will allow to get data for a Genome Wide Association Study (GWAS) and to search for genetic resistance to Xap in *Prunus armeniaca*, similar to the GWAS already carried out for the sharka virus.

ROGER

Project title: Genetic and genomic of the Rose resistance against Black Spot Disease

Acronym: RoGeR (Rose Genomic Resistance)

Project duration: 24 months - Start date: 01/09/2018 End date: 31/08/2020

Key-words: Rosa, Diplocarpon rosae, resistance genes, connected populations, association genetics, transcriptomic and comparative genomics

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Financial support from « Objectif Végétal »: 25k€

Summary:

Context

In the ornamental sector, the rose is the favourite flower of the consumers (local authorities and private individuals). In 2016, the French market of garden-roses contributed €57 million for a total of 4 millions of units sold essentially as grafted rose in pot or bare-root stock. To dump up the garden rose market, rose professionals created a "red label" that promotes roses with more vigour, floribundity and disease resistance. As the same time, use of pesticides has been forbidden in public gardens in France since the beginning of 2017 and will be also forbidden in 2019 for private gardens (J. Labbé and energy transition laws). Therefore, there has been a strong trend in demand by consumers to have resistant garden roses, and as a result, the development by breeders for roses that have a high level of resistance.

Black spot is the most important disease affecting rose aesthetics and was caused by *Diplocarpon rosae* (*Marssonina rosae* anamorph), an hemibiotrophic ascomycete fungus. Debener and Byrne (2014) estimated that there was only 7% of Rose with sustainable resistance into the US market.

Rose breeding is a long process (10 years on average) and requires the knowledge of sources of resistance. Several major genes and QTLs were located on genetic maps by research teams in Germany (University of Hannover) and in France (IRHS-GDO, Angers) (Malek & Debener, 1998; Marolleau et al, 2016; Shupert, 2005; Soufflet-Freslon et al, 2017; Whitaker et al, 2010; Whitaker & Hokanson, 2009).

Goals

The aim of this project is to develop complementary approaches (genetics, genomics, transcriptomics and comparative genomics) to characterise the genes involved in the rose resistance to black spot disease. The long-term objective is to develop new diagnostic markers to hasten the breeding process.

Methodology

For these objectives, we will take advantage of the different resources developed by the GDO team (interconnected progenies, reference genome sequence, and characterized collection of old garden roses, artificial inoculation protocol). Thanks to these resources, we will develop genetic (interconnected progeny to study QTL effects in different genetic backgrounds, candidate-gene association mapping), transcriptomic (comparison of transcripts during the interaction in susceptible and non-susceptible conditions) and comparative genomics (comparison of two pathosystems in the Rosaceae family). We will look for co-localization between genes detected in genetic approach (QTL), annotated genes and differentially-expressed genes.