PROJETS DEFIS SCIENTIFIQUES 2017

Thèses			
Acronyme	**Unité/ équipe porteuse**	**Coordinateur**	**Titre du projet /*Mots clés***
BRUTAL	IRHS-Emersys	Nicolas CHEN	Bean Resistance Using TAL effectors / *Bean, disease resistance, Xanthomonas, TAL effectors*
FALDIM	SONAS	Andreas SCHINKOVITZ	Formation of Apple-Phytoalexins and their detection by Laser Desorption Ionization and related Methods / *Apple, Phytoalexins, Matrix Assisted and Matrix Free Laser Desorption Ionization, Fingerprint Analysis, Resistance, Erwinia amylovora , Venturia inaequalis*
GESIIQUA	IRHS-QuaRVeg	Emmanuel GEOFFRIAU / Didier PELTIER	Genotype-EnvironmentS Interaction for Improving QUAlity and stability of carrot crop / *Carotenoid, genetics, biosynthesis pathway*
MECHARESIST	SiFCIR	Valérie RAYMOND	Identification of cellular and molecular resistance mechanisms to develop innovative strategies in pest insect control / *Pest insects - insecticide - resistance - nicotinic acetylcholine receptors*
ROSAROS	IRHS-ARCH'E	Alain VIAN	Implication of reactive oxygen species in the control of branching in plants/ *Reactive oxygen species – NADPH oxydase – Branching - Bud*
ROZRESIST	IRHS -GDO	Vanessa SOUFFLET-FRESLON	Genetics and genomics of rose resistance to black spot disease / *Rosa, Diplocarpon rosae, resistance genes, connected populations, association mapping, transcriptome, diversity*
SEEQUATOM	IRHS-Conserto	Olivier LEPRINCE	A systems biology approach to unravel the acquisition of seed quality during fruit ripening in tomato / *embryo, endosperm, gene network, locular gel, maternal environment, maturation, testa, transcriptomics, usable*
TRIANGLE	IRHS - ResPom	Charles-Eric DUREL (genetics) Marie-Noëlle BRISSET (PDS)	Triangular relationships between constitutive defenses, partial genetic resistance and efficiency of plant defense stimulators in apple / *Basal defenses, constitutive expression, genetic variation, partial resistance, plant defense stimulator, RT-qPCR, genome-wide association mapping, QTL mapping, expression QTL, apple, scab, fire blight*
Divin-Xf	*IRHS-Emersys*		*Emergence de Xylella fastidiosa en France : diversité des souches et routes d'invasion.*
Post-doc			
PREDIPATH	IRHS-Emersys	Marion FISCHER-LE SAUX	Design of marker sets and online tools to predict plant pathogenicity of bacteria / *Comparative genomics, predictive tool, genotypic markers, bacteria, plant pathogens*

Starters			
DIVIS	IRHS-Bioinfo	Julie BOURBEILLON	Biological Data Integration and Visualisation/ *Heterogeneous datasets, data integration, ontology, data visualisation*
EPICLONES	IRHS-ARCH'E	Nathalie LEDUC	ENVIRONMENTAL EPIGENETIC VARIANTS AS CLONAL MATERIAL IN HORTICULTURE/ *Urban horticulture, environmental stress, Epigenetic, vegetative propagation, mother-plants*
IONIS	IRHS-SMS	Françoise MONTRICHARD	Impact Of exogenous Nitrogen on early Seedling establishment of legumes / *Sustainable agriculture, agronomic context, Medicago truncatula, nitrate signal, primary root growth, early legume establishment, abscisic acid, reactive oxygen species, differential gene expression*
MORDOR	LBPV	Lucie POULIN	Seed-carried Microbiota from Orobanche during the early parasitic (Orobanche) cycle. Microbiote de l'Orobanche apporté par la graine à travers le cycle parasitaire/ *Metagenomics, microbiota, parasitic plant, Orobanche, diversity*

BRUTAL

Project title: Bean Resistance Using TAL effectors

Acronym: BRUTAL

Project duration: 36 months – Start date: 01/11/2017 End date: 31/10/2020

Key-words: Bean, disease resistance, Xanthomonas, TAL effectors

Coordinator: Chen Nicolas, IRHS / EmerSys, nicolas.chen@agrocampus-ouest.fr

PhD student : FOUCHER

Financial support from « Objectif Végétal »: 121 k€ (PhD grant from INRA / Région Pays de la Loire , - Running costs 25 k€ from Région Pays de la Loire)

Summary:

Context
The use of pesticides against crop bacterial diseases is very limited due to a lack of effective and non-toxic molecules. Thus, the most effective way to durably control bacterial pathogens is to combine prophylactic management with genetic selection of resistant cultivars. Bacteria from the genus *Xanthomonas* are responsible for Common bacterial blight of bean (CBB), the most devastating bacterial disease on bean.

Goals
The BRUTAL project aims at rapidly finding and deploying new sources of resistance to CBB. Our analyses will focus on characterizing novel functions targeted by *Xanthomonas* plant pathogens to promote virulence. A major goal of this project will be the construction of a battery of genome-edited bean plants carrying different resistances to CBB. These lines could then be used in seed mix and/or in rotation, enabling sustainable management of the disease.

Methodology
For this, we will analyse plant DNA regions targeted by TALs (Transcription Activator-Like) effectors injected in the plant cell by *Xanthomonas* bacteria. The diversity of these DNA regions will be analysed to search for natural sources of resistances usable by breeders. In parallel, we will modify these regions by genome-editing in bean cultivars and test if these modifications can lead to new resistances to CBB.

FALDIM

Project title: **F**ormation of **A**pple-Phytoalexins and their detection by **L**aser **D**esorption **I**onization and related **M**ethods.

Acronym**: FALDIM**

Project duration: 36 months – Start date: 1.10.2017 End date: 30.09.2020

Key-words: Apple, Phytoalexins, Matrix Assisted and Matrix Free Laser Desorption Ionization, Fingerprint Analysis, Resistance, *Erwinia amylovora* , *Venturia inaequalis*

Coordinator: Andreas Schinkovitz, SONAS, andreas.schinkovitz@univ-angers.fr

PhD student : Michaela SKOPIKOVA

Summary :

Context: Apple is an economically very important plant in modern agriculture and intensively treated with pesticides. Although very efficient in preventing diseases like apple scab and fire blight, the extensive use of pesticides causes a heavy environmental burden. Alternative concepts of plant protection therefore target the stimulation of the plant's immune system in order to fight infections. Phytoalexins are antimicrobial plant metabolites that are formed in response to infections and considered a key- factor in plants' immune defense. However the mechanisms of their formation as well as their chemical composition are barely explored. One reason for this lack of knowledge may be that conventional analytical methods (i.e. LC-ESI-MS) are unable to detect the totality of interesting compounds in a complex mixture.

Goals: The proposed project will apply, for the first time, a new analytical approach based on laser desorption ionization (LDI) to analyze infected apple tissues. Having a wider analytical window than conventional methods it is expected to identify new phytoalexins that are formed during infection process and to elucidate underlying metabolic mechanism. Eventually those compounds formed in sufficient yields will be isolated and used for further studies.

Methodology: Plants treated with an immune system activating agent (plant resistance inducers) as well as those exhibiting natural resistance and those of a susceptible control group will be all infected with apple scab and/or fire blight. Samples at different stages of the infection will then be analyzed by LDI but also by conventional analytical methods. Results will be compared in terms of the metabolomics profile of the three plant groups as well as in terms of the applied analytical methods. This will allow finding a well-adapted analytical strategy for a given sample set. Finally phytoalexins that are formed in large quantities will be isolated using chromatographic methods.

GESIIQUA

Project title: **Genotype-EnvironmentS Interaction for Improving QUAlity and stability of carrot crop**

Acronym: **GESIIQUA**

Project duration: 36 months – Start date: 01/12/2017 End date: 30/11/2020

Key-words: carotenoid, genetics, biosynthesis pathway,

Coordinator: Geoffriau Emmanuel, Peltier Didier, UMR 1345 IRHS, Team Quality and resistance to disseases in Vegetable crops (QuaRVeg),
Emmanuel.Geoffriau@agrocampus-ouest.fr
Didier.Peltier@univ-angers.fr

PhD student : Wilfried CHEVALIER

Summary :

Context – There is an increasing society concern about nutritional and organoleptic quality of fruits and vegetables. However, this trait is only poorly integrated in breeding programs, contrary to yield and disease resistance, due to the quality complexity both on genetic determinism and growing conditions effect points of view. Carrot is consumed worldwide and is the second most consumed vegetable in France. This economic importance combined with its role as an essential dietary source of provitamin A and society and breeders demands make carrot a model species for root crops to study the determinants of product quality elaboration. The main nutritional quality attribute of carrot is the carotenoid content, along with other compounds such as fibers, sugars, vitamins and minerals.

Goals –The genetic determinism of carotenoids has been studied recently, with mainly the involvement of carotenoid biosynthesis genes. However there are two main limitations: the approach targeting the biosynthesis pathway explains only partially the genetic determinism and the modulation of genetic factors by the environment is largely unknown. Some genotypes are more stable than others in this regard. The objective of the project is to identify the determinants of carotenoid content in carrot by evaluating the impact of environmental factors and the genotype X environment (GxE) effect, along with a non-targeted approach and, more globally, to understand the bases of genotype adaptability for nutritional traits which has not been studied so far.

Methodology - A panel of carrot material representative of the species diversity and varietal evolution will be studied in about 10 characterized environments over two years in the framework of the collaborative project CaroQual. The harvested lots will be characterized by the PhD for carotenoid (secondary metabolite) and sugar (primary metabolite as a comparison) contents. The weight of environmental factors will be evaluated by multiple regression analysis and GxE interaction effect by various analysis methods. Selected contrasted lots for GxE interaction and carotenoid content levels will be analyzed through a transcriptomic chip to identify the biosynthesis pathways and genes involved in differences of carotenoid accumulation and corresponding adaptability.

MECHARESIST

Project title: Identification of cellular and molecular resistance mechanisms to develop innovative strategies in pest insect control

Acronym: MECHARESIST

Project duration: 36 months – Start date: 2017/ 09/01 End date: 2020/ 08/31

Key-words: Pest insects - insecticide - resistance - nicotinic acetylcholine receptors

Coordinator: Pr Valérie RAYMOND, RCIM (SiFCIR), UPRES EA 2647 / USC INRA 1330 SFR 4207 QUASAV
valerie.raymond@univ-angers.fr

PhD student : BANTZ

Summary:

Context: Agriculture is facing an important challenge: it must be ensured that enough high-quality food is available to meet the needs of a continuously growing population. Even if chemical pesticides such as insecticides have been used successfully in controlling a number of pest insects, their uses, however, during several decades have lead to resistance emergence resulting in a decrease in the insecticide effects. Consequently and in accordance with Ecophyto 2 program, current and future agronomic production of food requires innovative solutions for existing and future changes and challenges, like counteract the setting-up of resistance of pest insects. Thus, it becomes necessary to consider the key cellular and molecular elements involved in the environmental adaptation of the insect such as the insecticide target conformation and calcium-dependent intracellular signaling pathways. Indeed, recent studies have demonstrated that the subunit composition of nicotinic acetylcholine receptors (nAChRs) could influence their affinity to insecticide neonicotinoids and the major role of calcium and calcium-dependent pathways in the modulation of insecticide responses in insects.

Goal: The aim of the project is to use for the first time the original insect model developed in the laboratory to explore how long it would take for an insect to adapt to chronic exposure to sublethal doses of neonicotinoid insecticide and how resistance factors are developed sequentially in this insect population during chronic exposure. Thus, this innovative project is not to study the mode of action of insecticides but to identify the cellular and molecular mechanisms developed by the insects for their environmental adaptation. We will focus our study on the characterization of cellular and molecular mechanisms, that include 1) the subunit composition of nAChRs, and 2) the intracellular signaling pathways involved in the modulation of the nAChR sensitivity to neonicotinoid and therefore involved in the modulation of the neonicotinoid efficacy. The insect model, the cockroach *Periplaneta americana,* which is a well-known suitable model to examine the influence of a chronic exposure to sublethal doses of insecticides will allow to better understand the resistance mechanisms involved 1) to develop innovative strategies in the circumvention of resistance to insecticides and 2) to improve the management of pest insect resistance. The knowledge obtained with these studies could be transposed to other insects such as the pea aphid to optimize pest control.

Methodology: Experiments will be carried out on adult dorsal unpaired median (DUM) neurons isolated from the central nervous system of *P. americana* cockroaches exposed or not to a sublethal dose of the neonicotinoid, imidacloprid during 30 days. Electrophysiological studies, using the patch-clamp technique, combined to the single cell RT-PCR technique, will bring   new correlated insights between electro-pharmacological profile of nAChRs and their subunit composition. Then, we will determine in heterologous system (Xenopus oocytes) if the composition of nAChR subunits modulates the amplitude of the current induced by imidacloprid and therefore the sensitivity of nAChRs to insecticides. Moreover, the identification of calcium- dependent intracellular signaling pathways including kinases or phosphatases involved in increase of the sensitivity of nAChRs to insecticide will be performed by single cell RT-PCR. Finally, using calcium imaging technique combined with the technique of RNA interference, we will be able to block the previous identified kinases or phosphatases and to determine their involvement in this modulation.

ROSAROS

Project title: Implication of reactive oxygen species in the control of branching in plants.

Acronym: ROSAROS

Project duration: 36 months – Start date: 01/10/2017 End date 30/09/2020

Key-words: Reactive oxygen species – NADPH oxydase – Branching - Bud

Coordinator: VIAN Alain, IRHS / ARCH-E, alain.vian@univ- angers.fr

PhD student : Alexis PORCHER

Summary:

Plant shape is an important parameter for consumer choice of ornamental plants. Nowadays, the preferences are oriented towards compact and highly ramified plants that better fits urban life mode. These plants require several pruning and/or chemical treatments to obtain the requested shape. These treatments are costly and not environmental friendly. Thus, alternative methods to obtain such plant shape are under investigation. In rose bush, bud outgrowth that control plant shape could be modulated by environmental factors such as light or nutrition. The precise understanding of the bud outgrowth mechanism appears as a critical step to develop alternatives based on environmental factors to pruning and chemical treatments. In the present project, we propose to append the existing molecular bud outgrowth model based on light/hormones/nutrients interactions with the contribution of oxidizing metabolism that is recently proposed to play an active role in plant development, including bud outgrowth.

ROZREZIST

Project title: Genetics and genomics of rose resistance to black spot disease

Acronym: RozResist

Project duration: 36 months – Start date: 01/10/2017 End date: 30/09/2020

Key-words: *Rosa*, *Diplocarpon rosae*, resistance genes, connected populations, association mapping, transcriptome, diversity

Coordinator: Soufflet-Freslon Vanessa, IRHS – GDO, vanessa.soufflet-freslon@agrocampus-ouest.fr

PhD student : Diana LOPEZ-ARIAS

Financial support from « Objectif Végétal »: 96 k€ (PhD grant INRA / Région Pays de la Loire)

Summary:

Black spot (caused by the fungal pathogen *Diplocarpon rosae*) is the most severe disease of roses in the outdoor landscape. Most cultivars are susceptible to this disease and its control requires many fungicide treatments. Pathogen attacks have a serious impact on aesthetic quality. It is now crucial to reduce such use of pesticides in ornamental cultures for production cost decrease and also for environmental and consumer health reasons. In this way, use of pesticides will be forbidden in public and private gardens in France in 2017 and 2019, respectively (J. Labbé law). Growing genetically resistant rose cultivars should provide an alternative to chemical control of the disease. Genetic studies have revealed both qualitative resistance conditioned by dominant genes and quantitative (partial) resistance conditioned by multiple genes. In different pathosystems, qualitative resistance is known to be race specific and compromised quickly through pathogen mutation or migration while quantitative resistance is assumed to be race non-specific and could be more durable. A few rose resistance QTL to black spot were identified in only one rose genotype. Here we propose to investigate both genetic and genomic bases of disease resistance. First, we will identify QTL, effective in different genetic backgrounds and against a range of pathogen isolates. Multiparental connected populations will be scored for black spot resistance after both artificial inoculations and natural infections. This project aims also to characterize the candidate-genes underlying resistance QTL that are identified, thanks to positional and transcriptomic approaches. Finally, we will try to validate by association mapping the best candidate-genes previously identified. This will allow to evaluate the efficiency of resistance genes in wider genetic background and to identify different favorable alleles for a same locus. In the end, this thesis aims to supply some sources of genetic resistance that will be used for rose breeding.

SEEQUATOM

Project title: **A systems biology approach to unravel the acquisition of <u>seed</u> <u>quality</u> during fruit ripening in <u>tom</u>ato**

Acronym: **SEEQUATOM**

Project duration: 36 months – Start date: 1/11/2017 End date: 31/10/2020

Key-words: embryo, endosperm, gene network, locular gel, maternal environment, maturation, testa, transcriptomics, usable plantlets, vigour

Coordinator: LEPRINCE Olivier, IRHS/Conserto, olivier.leprince@agrocampus-ouest.fr

PhD student : Elise BIZOUERNE

Financial support required from « Objectif Végétal »: 115,6 k€ (PhD grant Agrocampus-Ouest / Région Pays de la Loire – Running costs : 25 k€ Région Pays de la Loire)

Summary:

Seed vigour is an estimate of how successfully a seed lot will establish seedlings under the wide range of environmental conditions. Seed companies invest considerable resources to produce seeds of high vigour. However, this is very difficult to achieve because seed vigour is a multifactorial trait that is determined by complex gene by environment interactions. Seed vigour traits are sequentially acquired during development via various genetic programs that are poorly understood. In addition, there is increasing awareness that environmental cues perceived by both the mother plant and the developing zygote have dramatic effects on seed quality. Therefore, there is an urgent need for an integrative vision of the elaboration of seed vigour during maturation. This is especially important for high-value seeds such as tomato, a key strategic species for the seed industry being the first source of vegetable worldwide. The aim of the project is to uncover the regulatory genes controlling the acquisition of seed vigour during tomato fruit maturation using a systems biology approach. Along with the physiological characterization of germination and seedling emergence, we will generate detailed tissue-specific transcriptomic maps of seed development subjected to different environments. Gene network approaches and innovative multivariate analyses will be used to enable the identification of key regulatory genes that correlate with seed quality. Also, we will explore how the fleshy fruit tissues surrounding the seeds influence the acquisition of seed vigour.

TRIANGLE

Project title: Triangular relationships between constitutive defenses, partial genetic resistance and efficiency of plant defense stimulators in apple

Acronym: TRIANGLE

Project duration: 36 months – Start date: 01/11/2017   End date: 31/10/2020

Key-words: basal defenses, constitutive expression, genetic variation, partial resistance, plant defense stimulator, RT-qPCR, genome-wide association mapping, QTL mapping, expression QTL, apple, scab, fire blight

Coordinators: Durel Charles-Eric (genetics) Brisset Marie-Noëlle (PDS), IRHS – ResPom
charles-eric.durel@inra.fr
marie-noelle.brisset@inra.fr

PhD student: BENEJAM

Summary:

Context: Apple is susceptible to numerous diseases and pests that request intensive chemical controls with much concern for human health and environment. Breeding new resistant varieties and/or stimulating plant defenses in commercial orchards are thus major stakes. Especially, increasing the level of basal defenses against a large range of bioagressors instead of focusing on complete resistance against particular ones is a major goal to efficiently decrease the number of treatments. We hypothesize that the constitutive level of basal defenses (i.e., before any induction) is driving the plant capacity to reach a sufficient resistance level to control a pathogen or pest attack, possibly with the help of Plant Defense Stimulators (PDS). The present PhD project will explore the triangular dependencies between constitutive defenses, partial genetic resistance and efficiency of plant defense stimulators in apple scab and fire blight which are two main diseases in commercial orchards. It will partly rely on the RFI-OV-funded project 'PREDIRE' (2016-2019) which aims at exploring the genetic variation of constitutive defenses thanks to expression QTL (eQTL) analysis, and on a supplementary QTL analysis of apple reactivity to PDS. It will also benefit from the 'FALDIM' project ("Formation of Apple- phytoalexins and their detection by laser desorption ionization and related methods") submitted to RFI-OV by SONAS +ResPom. The genomic resources recently developed in apple at IRHS Angers, the germplasm resources present on-site, and the skills of the involved teams in bioanalysis, quantitative genetics and functional analysis of defenses with or without PDS will allow the PhD student to efficiently develop this project.

Goals: The PhD student will address three sets of research questions:

1- What is the extent of the genetic variation of apple constitutive defenses? Can this variation be used as a predictor for partial genetic resistance to apple scab and fire blight?

2- What is the extent of the genetic variation of PDS efficiency in apple? Is this efficiency related to the levels of constitutive defenses and/or partial genetic resistance?

3- What are the genetic determinisms of apple constitutive defenses and PDS efficiency? Can we associate these determinisms with partial resistance determinism, candidate genes and possibly metabolites?

Methodology:

- Analysis of the constitutive expression of selected defense-related genes thanks to RT-qPCR using consensus primers designed to be conserved over a large core collection of apple cultivars
- Phenotyping of scab and fire blight resistance of the apple cultivars and of a F1 progeny preliminary treated or not by the PDS Bion and further inoculated under controlled conditions (greenhouse)
- GWAS (Genome-Wide Association Studies) and detection of eQTLs in the core collection + QTL analysis in a F1 progeny for apple reactivity to PDS.

PREDIPATH

Project title: Design of marker sets and online tools to predict plant pathogenicity of bacteria

Acronym: PREDIPATH

Project duration: 24 months – Start date: 30/10/2017 End date: 16/03/2019

Key-words: comparative genomics, predictive tool, genotypic markers, bacteria, plant pathogens

Coordinator: FISCHER-LE SAUX Marion, UMR1345 – Emersys, Marion.le-saux@inra.fr

Post-doctorante : Felipe LIRA

Summary:

Plant pathogenic bacteria are responsible for huge socio-economic impact in agroecosystems. Whereas sustainable plant health management requires accurate identification of the pathogens their identification is not straightforward. This is even more challenging when non-symptomatic samples are analyzed, for instance during sanitary control of plants and seeds or during ecological studies aiming to decipher the ecological niches of plant pathogens. Furthermore, development of biotechnology based on microbial resources (including biocontrol) leads to an increased demand for evaluation tools that could predict the safety of selected microorganisms for plants. An equivalent need emerge from user satisfaction surveys of the French collection of associated bacteria (CIRM-CFBP) whose users are eager to precisely know the pathogenic status of preserved strains.

The aim of this project is to take advantage of the numerous whole genome sequences of plant pathogenic bacteria available to identify genetic and genomic markers of plant pathogenicity and to develop web tools that could help to predict if a given bacterial isolate could be a plant pathogen only based on its genome. In the future, such a web tool could replace, in part, time and cost consuming pathogenicity assays by restricting these tests to ambiguous results.

First, datasets composed of plant pathogenic bacteria and their commensal relatives will be set up. Comparative genomic analyses between these two kinds of bacteria will allow deciphering the gene content and genome traits characteristics of plant pathogens. Association analysis using statistical and correlation tests will be performed to link genotype features to the pathogenic profile of the strains. A blind test will allow evaluating the performance of the method. Pipelines and web tools developed in the frame of this project will be available on a web-based portal for plant pathologists and diagnosticians.

DIVIS

Project title: Biological Data Integration and Visualisation Acronym: DIVIS

Project duration: 36 months – Start date: 01/01/2018 End date: 31/12/2020

Key-words: heterogeneous datasets, data integration, ontology, data visualisation

Coordinator: Julie Bourbeillon, IRHS / Bioinfo, julie.bourbeillon@agrocampus-ouest.fr

Summary:

In biology high-throughput techniques and the multiplication of databanks have caused the scale at which data are acquired and shared to increase exponentially in recent years. The field is entering the so called "Big data" era. However, the datasets accumulated by biologists during the course of their experiments are often heterogeneous. Moreover, even if they can be stored and retrieved together through databanks they are usually not bound together because there are discrepancies between the experimental settings. This poses problems to biologists to analyse jointly these disparate datasets in order to acquire new insights into their biological interpretations.

However existing software does not provide complete user-friendly solutions to gather these datasets together, render their size manageable for instance through data summaries and allow scientists to interpret them easily through visual displays. The goal of the project is therefore to perform a preliminary study and explore new bioinformatic approaches to solve this issue in a generic way, that is to say not tailored for a specific organism or a limited range of experimental data.

During the course of the project we will develop a prototype software using apple and seed datasets. The tool will manipulate a large matrix containing all datasets extracted from the research unit database and: (I) normalise datasets so that they can be treated jointly; (ii) group similar samples analysed in similar experimental settings. The grouping process will be performed based on previous knowledge stored in specifically designed ontologies; (iii) represent each group by a representative archetype individual; (iv) summarise data for the archetype individuals; (v) build a visual display of the data summaries which the biologist will be able to navigate to acquire an understanding of the underlying datasets.

EPICLONES

Project title: ENVIRONMENTAL EPIGENETIC VARIANTS AS CLONAL MATERIAL IN HORTICULTURE

Acronym: EPICLONES

Project duration: 24 months – Start date: 01.10.2017   End date: 30.09.2019

Key-words: Urban horticulture, environmental stress, Epigenetic, vegetative propagation, mother-plants

Coordinator: LEDUC Nathalie, ARCH'E-IRHS, Nathalie.Leduc@univ-angers.fr

Summary:

Context: Crop adaptation to stress-inducing environment is a key issue in the general context of climatic change but also to face the increasing demand for plants adapted to urban adverse growth conditions. Genetic selection of new varieties through crossing of parental lines is the classical way to improve plants, but it is a long process. New methods for crop improvement are therefore search for.

Goals: The aim of the Epiclones project will be to assess whether plants can be acclimated to an adverse environment through prior culture of successive vegetative generations under stressful conditions and selection of the best resistant plants at each generation. The reasoning behind is that stress may imprint epigenetic changes in plant genome that may remain stable through vegetative propagation and confer plants a better and long lasting adaptation to adverse growth conditions. The goal of the project will then be to assess whether such acclimation and epigenetic changes can occur through such a strategy and bring about increased resistance.

Methodology: Petunia "star" variety will be cultured under hydric stress condition (one of the most important stress in urban environment) and successive cutting-derived generations cultured similarly. Performances of the different generations to resist hydric stress will be evaluated through several phenotypic characters, physiological and molecular analysis and compared to performances of the initial mother-plants and of clones that were propagated under no stress. Petunia plants will also be cultured *in vitro* in the presence of a de- methylating agent, known to induce epigenetic changes in genomes and subjected to hydric stress using polyethylene glycol-rich medium. These *in vitro* plants will then also be examined concerning their resistance to hydric stress. The study of the global level of DNA methylation as well as of the methylome sequences of some of the Petunia plants that were subjected to hydric stress both in the growth chamber and *in vitro*, will finally allow to determine whether correlations exist between epigenetic changes and plant acclimation to hydric stress, as well as to assess the stability of such epigenetic changes through successive clonal generations.

IONIS

Project title: Impact of exogenous nitrogen on early seedling establishment of legumes in the context of sustainable agriculture Acronym: IoNiS (Impact Of exogenous Nitrogen on early Seedling establishment of legumes)

Project duration: 36 months – Start date: 01.11.2017; End date: September 31.10.2020

Key-words: sustainable agriculture, agronomic context, *Medicago truncatula*, nitrate signal, primary root growth, early legume establishment, abscisic acid, reactive oxygen species, differential gene expression

Coordinator: Montrichard Françoise, UMR 1345 IRHS, SESaN and BioInfo teams
francoise.montrichard@ univ-angers.fr

Financial support from « Objectif Végétal »: 25 k€ (Région Pays de la Loire)

Summary:

Context: Legumes play an important role in human and livestock alimentation due to their high level of proteins in seeds and aerial parts used as forage. Legumes by fixing atmospheric N2 also take part to a significant ecosystemic service in cropping systems contributing to nitrogen enrichment of soils and thereby to sustainable agriculture. While many studies on legume biology are focusing on symbiotic nitrogen fixation processes, our team aims to characterize the early post-germinative processes leading to successful legume establishment taking into account the impact of exogenous nitrate or abiotic stresses. Primary root growth is crucial for successful seedling establishment. It depends on nutrient availability and notably on the level of nitrate content in the soil which varies greatly. This is also true for legumes during early seedling development, when the fixation of atmospheric N2 is not in place. However, nitrate is not only a nutrient but also a signal involved in the regulation of primary root growth. At early stage, we showed in *Medicago truncatula*, a model legume, that nitrate can inhibit root growth.

Goal: The goal of this project is to decipher the nitrate signaling pathway that leads to a reduction of primary root growth in *M. truncatula* by the restriction of cell elongation. We have recently found that the nitrate transporter MtNPF6.8 acts as a nitrate sensor involved in the response to nitrate, mutants deficient in MtNPF6.8 (*npf6.8*) being insensitive to nitrate. How the signal, perceived by the transporter, is transduced is still not known. However, the fact that abscisic acid (ABA), a vegetal hormone, restored the inhibitory effect of nitrate in *npf6.8* mutants suggests that ABA is involved in the signal transduction. Reactive oxygen species (ROS) are also likely to play a role in the mediation of the nitrate signal because they govern root growth through cell division and elongation. Thus, nitrate should modify the ROS balance in roots. Because both ABA and ROS are known to control gene expression, nitrate signal may also alter gene expression through these two mediators. We want to determine how nitrate modifies the ROS balance and identify the genes mediating the nitrate signal. A special interest will be given to genes encoding redoxins or transcription factors possibly redox regulated, all being good candidates to mediate the signal downstream as well as to other target genes to gain clues on the mechanism underlying the reduction of root growth through the function of the genes identified. We want to develop within the IRHS unit a novel competence for the integration of the data at the two levels of gene expression and unravel post-transcriptional regulation events.

Methodology: We will mainly use two genotypes contrasting for nitrate sensitivity, a wild type genotype sensitive to nitrate (R108) and the *npf6.8* mutant in the same genetic background, which is deficient in a nitrate sensor and has lost the nitrate sensitivity. The impact of nitrate on ABA or ROS accumulation in different parts of the root will be assessed. The incidence of a modification of ABA signaling or ROS content on root growth, will also be determined through a pharmacological approach or a reverse genetic approach using mutants affected in ABA signaling or ROS production. In parallel, the impact of exogenous nitrate or ABA on gene expression in the wild type and in the *npf6.8* mutant lines will be determined by both transcriptomic and proteomic studies. We will work in synergy with experts in transcriptomics and bioinformatics (present locally) and proteomics (Paris Saclay).

MORDOR

Project title: Seed-carried **M**icrobiota from **Or**obanche **d**uring the early parasitic (**Or**obanche) cycle. Microbiote de l'Orobanche apporté par la graine à travers le cycle parasitaire.

Acronym: MORdOR

Project duration: 24 months – Start date: 01/09/2017 End date: 31/08/2019

Key-words: metagenomics, microbiota, parasitic plant, Orobanche, diversity

Coordinator: Lucie Poulin, LBPV, lucie.poulin@univ-nantes.fr

Summary:

Holoparasitic weeds of the Orobanchaceae family – or broomrapes- are of great concern for a number of cultivated crop species in France including rapeseed, tobacco, melon and sunflower. Especially in western France, *Phelipanche ramosa* broomrape species is expending every year on rapeseed crop fields. No gene of resistance has been yet found and breeders struggle to produce sustainable varieties of crops that are less susceptible to those parasites. Moreover no treatment is available beside agricultural approaches such as crop rotation. However, parasitic seeds are produced in large quantities and remain in soils for years, which can result in devastating infestations when climatic conditions allow it. In the last decade, studies on host --- parasite rhizospheric interactions have unravelled certain aspects of the parasitic cycle. It is well established that parasitic seeds of *P. ramosa* germinate in response to stimulants of germination (SGs) such as strigolactones or isothiocyanates (degradation products of gluscosinolates) that are present in the rhizosphere and are contained most largely in plant root exudates. Besides, microbiota is likely to contribute largely to the parasitic plant development.

The MORdOR project aims to participate in the global comprehension of the parasitic overall diversity and host range and in an original way with a focus on microbial communities. More particularly, the research project focuses exclusively on the parasitic seed-carried microbiota and intends to characterize *P. ramosa*- carried microbial communities depending on its genotype and upon host type stimulation for germination.

Metabarcoding methods will be used for characterizing bacterial and fungal diversity and communities. In a first approach, seed-carried microbiota from different representative *P. ramosa* genotypes will be characterised and compared. In a second approach, the variation of microbial communities of the same parasitic seeds upon interaction with different host will be evaluated.