PROJETS DEFIS SCIENTIFIQUES 2019

Thèses			
Acronyme	Unité - équipe porteuse	Coordinateur	**Titre du projet /*Mots clés***
FlaResCAd	IRHS - QuaRVeg	Mathilde BRIARD / Latifa HAMAMA	Flavonoids and resistance of Carrot to *Alternaria dauci* / *Leaf blight, Quantitative resistance, Carrot, Secondary metabolites, UHPLC-ESI-MS^n, CRISPR/Cas9 genome editing*
SPADES	IRHS - BIDefI	Sébastien AUBOURG / Jean-Pierre RENOU	Functional characterization of a new family of secreted peptides involved in defense and development control in *Arabidopsis* / *hormonal peptide, phytocytokine, ROS detoxification, defense signaling, root development, pathogen resistance*
TEXT-EAU-TERREAU	EPHor	Jean-Charles MICHEL	Substrate Texture: A unifying approach to explain and control Physical Properties and Water Efficiency in Horticultural Substrates / *particle size, particle shape, structure, hydrophobicity, water retention*
UPROAR	SONAS	Pascal RICHOMME	In silico-guided design of semisynthetic antifungal derivatives targeting the Unfolded Protein Response (UPR) pathway: Towards an alternative crop protection / *plant pathologies, phytochemistry*
Post-doc			
ECLENUS	IRHS - SMS	Françoise MONTRICHARD	Exploring new ways to control Legume nitrate utilization and signaling / *sustainable agriculture, agronomic context, Medicago truncatula, nitrate signal, primary root growth, early legume establishment, reactive oxygen species, differential gene expression, metabolomics, fluxomics*
epiDT	IRHS - Conserto	Jérôme VERDIER	Deciphering the molecular switch of seed desiccation tolerance to improve plant stress tolerance / *chromatin conformation, epigenetics, desiccation tolerance, seeds, stress*

Starters			
THERMIT	IRHS - SMS	David MACHEREL	Building subcellular thermometers to study plant mitochondria self-warming in the context of cold tolerance / *mitochondria, cold tolerance, freezing tolerance, thermosensor*
TRANSMISSION	IRHS - EmerSys	Armelle DARRASSE	Transcriptome profiling of plant-bacterial interactions during seed transmission / *Seed transmission, seed development, transcriptomes, methylomes, Phaseolus vulgaris, Xanthomonas citri pv. fuscans*

FlaResCad

Project title: Flavonoids and resistance of Carrot to *Alternaria dauci*

Acronym: FlaResCAd

Project duration: 36 months - Start date: 01/10/2019 End date: 30/09/2022

Key-words: Leaf blight, Quantitative resistance, Carrot, Secondary metabolites, UHPLC-ESI-MS^n, CRISPR/Cas9 genome editing

Coordinators: Mathilde BRIARD, Latifa HAMAMA / IRHS – QuaRVeg
mathilde.briard@agrocampus-ouest.fr;
latifa.hamama@agrocampus-ouest.fr

PhD student : Lauriane GILLY

Financial support from « Objectif Végétal »: 19 k€ (Région Pays de la Loire)

Summary:

Alternaria dauci is a major disease threatening carrot, a worldwide important vegetable food. Resistance of carrot to *Alternaria dauci* is a critical tool in the context of agroecology but a complex and quantitative trait. Previous works highlighted numerous quantitative trait loci for resistance (rQTL) to *Alternaria* leaf blight and potential mechanisms underlying some of these rQTL. Flavonoids were proposed to be linked with the mechanism underlying the major one (rQTL on chromosome 6 with R^2>20%). The combination of genetic, metabolomic and transcriptomic approaches led to the identification of two genes as candidates responsible for the accumulation of these flavonoids. The goals of the present thesis project are to decipher the role of flavonoids in resistance and to evaluate the control of their accumulation by the two candidate genes. Questions to be answered are: i) can the candidate genes modulate the level of resistance in resistant and susceptible genotypes? ii) how the flavonoid accumulation is regulated by the candidate genes expression? iii) do the candidate flavonoid compounds have a direct effect on the pathogen? iv) could these metabolites be useful as resistance early detection tools for breeders? In a proof of concept approach, the PhD student will address the first two questions through over/under-expression of the candidate genes with *Agrobacterium tumefasciens* transformation and with CRISPR/Cas9 genome editing system. The impact of over- or under- expressed candidate genes on plant protection against *A. dauci* will be evaluated on transformed regenerated plants. Both types of transformants will also be characterized for their flavonoid profile by UHPLC-ESI-MS^n. To optimize this last tasks, a preliminary analysis of the influence of plant stage on flavonoid accumulation will be realized. For this, UHPLC-ESI-MS^n quantification of flavonoids will be performed every two weeks from seedling to adult development stage in resistant and susceptible genotypes. As mentioned above, another goal of this work is to evaluate the direct impact of flavonoid compounds on the pathogen and determine if they are defence molecules exhibiting an antifungal activity or if they are markers of the carrot resistance to *A. dauci*. This task will require sufficient amount of pure flavonoids. Due to the concentration of these secondary metabolites in carrot leaves, sourcing issues are a concern. They will be dealt through a thorough survey of the literature to determine the natural resource(s) producing higher amounts of one (or more) specific flavonoid heteroside candidate(s) previously identified, followed by a classical three-step strategy involving extraction of plant materials, purification of the corresponding extracts and analytical characterization of the pure natural products. Carrot may also produce other secondary metabolites as defence tools in disease resistance. To shed light on these aspects, extraction of the leaves from both resistant and susceptible plants, inoculated or not, will be achieved and their analytical profiles will be compared to access qualitative and quantitative data. The toxicity of the extracts will be evaluated on the fungus by means of a nephelometry approach. A bioguided fractionation strategy will be undertaken to identify, purify and characterize potentially active compounds from carrot foliar extracts whose toxicity may correlate with the difference between resistant and susceptible plants. The PhD student will develop an integrated view of the role of flavonoids and related compounds in resistance to *A. dauci* and propose tools for carrot breeding.

SPADES

Project title: Functional characterization of a new family of secreted peptides involved in defense and development control in *Arabidopsis*

Acronym: **SPADES** (**S**COOP **PA**thway in **DE**velopment and **S**tress)

Project duration: 36 months - Start date: 01/10/2019 End date: 30/09/2022

Key-words: hormonal peptide, phytocytokine, ROS detoxification, defense signaling, root development, pathogen resistance

Coordinators: Sébastien AUBOURG, Jean-Pierre RENOU / IRHS – BIDefi
sebastien.aubourg@inra.fr
jean-pierre.renou@inra.fr

PhD student : Marie-Charlotte GUILLOU

Financial support from « Objectif Végétal »: 17, 4 k€ (Région Pays de la Loire)

Summary:

Context:
Plant responses to pests and pathogens are coordinated by regulatory proteins and hormones (Buscaill and Rivas, 2014). Among these actors, small secreted peptides, also named peptide phytohormones or phytocytokines, may directly interact with pathogens or act in signaling and cell-to-cell communication (Murphy *et al*., 2012; Gust *et al*., 2017). However, only a small fraction of the gene space liable to encode such signaling peptides has been described and their structural and functional diversity appears to be largely underestimated (Matsubayashi *et al*., 2014). Therefore a huge part of the whole array of plant genetic defenses is still unexploited. In this context, by combining meta-analysis of transcriptomes, bioinformatics predictions and experimental assays on mutants and synthetic peptides, we have identified a gene family of 14 paralogs in *Arabidopsis thaliana* encoding precursors of putative secreted peptides, hereinafter referred as SCOOP. We have already shown that among them, SCOOP12 is involved in the control of defense pathway and the root elongation through the regulation of reactive oxygen species (ROS) responses (Gully, Pelletier *et al*., 2019).

Goals:
The PhD project aims at deciphering the signaling pathway triggered by the SCOOP12 peptide and to enlarge the functional characterization to the 13 other SCOOP12 paralogs identified in the Arabidopsis genome. These goals require the identification of functional partners and especially the transmembrane receptor which interact directly with the mature peptide. Our first results provide us the preliminary backbone of a downstream cascade such as the involvement of the *BAK1* as a co-receptor and the induction of the phospholipid pathway (Gully, Pelletier *et al*., 2019) but numerous gaps remain to fill. Furthermore, the exact processing of SCOOP12 is based on structural comparisons and stay to be experimentally confirmed. Besides its role in plant defenses, we also aim to prove our hypothesis regarding the role of SCOOP12 as a moderator of root elongation, possibly through H_2O_2 level control.

Methodology:
The work, conducted on *A. thaliana* plants and cell suspensions, will include complementary approaches in the fields of functional genomics (genotyping, qPCR, cloning…), cytology (subcellular localization of the peptides), and biochemistry (enzymatic assays, immunoprecipitations, ROS species quantitation). It will allow studying the impact of mutations and peptide application on plant defenses and development, and validate putative players of the SCOOP pathway. Already established collaborations with experts in and outside IRHS will ensure efficient supervisory and formation of the PhD student for the successful achievement of all these goals.

TEXT-EAU-TERREAU

Project title: Substrate Texture: A unifying approach to explain and control Physical Properties and Water Efficiency in Horticultural Substrates

Acronym: **TEXT-EAU-TERREAU**

Project duration: 35 months - Start date: 01/11/2019 End date: 30/09/2022

Key-words: particle size, particle shape, structure, hydrophobicity, water retention

Coordinator: Jean-Charles MICHEL / Agrocampus Ouest – EPHor

jean-charles.michel@agrocampus-ouest.fr

Financial support from « Objectif Végétal »: 30k€ (Région Pays de la Loire)

Summary:

Context
In soilless culture, improving water efficiency is of vital importance to avoid risks of root asphyxia, nutrient leaching, infestation by pathogens in case of excessive irrigation, and conversely, risks of substrates hydrophobicity and plant physiological stress in driest conditions. Water efficiency closely depends on the physical and hydraulic properties (water retention and flow, ability to drain) of the substrates, themselves resulting of their particles size and shape (texture) and organization (structure).
Texture is considered as the main reference describing a soil and many of its properties, *i.e.* its « Identity Card », and then is routinely characterized. In contrast, texture has never been studied for horticultural substrates, in part because its analysis is much more complex due to the large diversity of particles sizes and shapes (fibers, chips, etc.) used in horticultural substrates, in comparison with mineral soils which are mostly granular particles. These irregular shapes fall together to create a pore size distribution much larger than in mineral soils (over 75% by volume), so that particles size and shape greatly influence the resulting matric structure of the materials, and therefore their physical and hydraulic properties.

Goals
The general scientific aims of this project are (1) to finely analyze the texture (particles size and shape) of a large diversity of substrates components (peats, barks, wood products, coir, etc.), (2) to understand its influence on their structure, and then (3) to identify some relevant parameters from texture and structure used to develop a model for explaining, predicting and precisely controlling physical and hydraulic properties of substrates. By using this approach, this project would contribute to considerable scientific and industrial breakthroughs, improve water use and efficiency in soilless culture, and likely redefine the composition selection (raw materials, fractions, mixes) of substrates. This would also favor sustainable peat alternatives, as both describing physical properties required and irrigation techniques needed by the end-users. So that this project will result in more sustainable horticultural soilless systems.

Methodology
This project is based on the use of a very innovative and original technique for characterizing particles size and shape of substrates' components using dynamic image analysis. Other intrinsic properties such as the specific surface area and surface properties (hydrophobicity) will complete this detailed analysis of texture. These data will be coupled with a mechanistic description of the porosity (pore size, shape and connectivity) using X-ray microtomography, then with the measurements of rewetting capacity and other physical and hydraulic properties of substrates (water and air retention and flows). A unifying model will be then developed in order to explain, predict and control substrates' properties from both texture and structure analysis.

UPROAR

Project title: *In silico*-guided design of semisynthetic antifungal derivatives targeting the Unfolded Protein Response (UPR) pathway: Towards an alternative crop protection

Acronym: **UPROAR** (Unfolded Protein RespOnse xAnthone cRop protection)

Project duration: 36 months - Start date: 01/10/2019 End date: 30/09/2022

Key-words: plant pathologies, phytochemistry

Coordinators: Pascal Richomme / UA – SONAS
pascal.richomme@univ-angers.fr

PhD student: Thomas CHARPENTIER

Financial support from « Objectif Végétal »: 110 k€ (Région Pays de la Loire _ Angers Loire Métropole)

Summary:

Endoplasmic reticulum is an important cell compartment for protein synthesis and its quality control. During proteogenesis, various biotic, abiotic, or physiological constraints can result in homeostasis imbalance, leading to an accumulation of (un)(mal)formed proteins. To cope with this imbalance, eukaryotes trigger a physiological adaptive response —so-called UPR (Unfolded Protein Response) pathway— in order to ensure the maintenance of homeostasis and cell survival. In mammals, the response to this stress involves the activation of three main effectors *i. e.* the transmembrane proteins PERK, ATF6 and IRE-1. However, in other organisms, such as fungal phytopathogens, IRE1 represents the only effector of the UPR pathway. Previous works showed that this pathway is involved in virulence by promoting the protection of pathogenic fungi against the toxicity of plant defense metabolites and in particular against phytoalexins.

Towards a planned reduction of the use of agrochemicals in crop protection, the present work will aim to develop new and efficient inhibitors of the fungal phytopathogens UPR pathway. We will favor natural inhibitors to be applied at low doses and acting in synergy with natural plant defense metabolites in order to prevent or reduce the pathogen growth.

These inhibitors will be based, after *in silico* screening, on natural xanthones (dibenzo gamma-pyrone scaffold) and chemical modifications will be *in silico*-guided. Xanthones will be obtained from natural and renewable sources such as mangosteen pericarps [harvested from mangosteen tree fruits, *Garcinia mangostana* (*Clusiaceae)*]. Optimized semisynthetic derivatives will be developed, and their ability to bypass the UPR-related defense mechanism of fungal phytopathogens as well as to restore the effectiveness of the natural defenses of crops will be evaluated.

ECLENUS

Project title: **Exploring new ways to control Legume nitrate utilization and signaling** / Explorer de nouvelles voies pour contrôler la signalisation et l'utilisation du nitrate

Acronym: **ECLENUS**

Project duration: 19 months - Start date: 01/10/2019 End date: 30/04/2021

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

Coordinator: Francoise Montrichard / IRHS – SMS
francoise.montrichard@univ-angers.fr

PhD student: Lukasz Pawel TARKOWSKI

Financial support from « Objectif Végétal »: 95,5k€ (Région Pays de la Loire _ Angers Loire Métropole

Summary:

Context

Legumes play an important role in human and livestock diet due to their high protein content in seeds and aerial parts (which can be used as forage). Legumes also have a key role in cropping ecosystems by fixing atmospheric N_2 thereby contributing to nitrogen enrichment in soils and thus to sustainable agriculture. Primary root growth is crucial for successful seedling establishment and depends on nutrient availability such as soil nitrate concentration, which is rather variable. Legumes do require nitrate during early seedling development since atmospheric N_2 fixation is not functional yet. That said, nitrate is not only a nutrient but also a signal involved in the regulation of primary root growth. We have recently shown that contrary to model plants like Arabidopsis where moderate nitrate concentration stimulates primary and lateral root growth, in legumes nitrate inhibits primary root growth at early developmental stages (model plant *Medicago truncatula*). **This represents a significant change in paradigm for nitrogen nutrition**: First, since soil nitrate concentration varies spatially in the field and can reach high values locally, this may explain why legume cultivation leads to considerable heterogeneity in seedling establishment; Second, since nitrate itself inhibits its own utilisation, this should in principle lead to a vicious circle whereby nitrate leaching and aquifer pollution is favoured over its consumption by legumes.

Goal:

The project **ECLENUS** aims to open avenues to manipulate nitrate signaling so as to improve seedling homogeneity and ability to capture soil nitrate. Solving this question is crucially important because legume seedlings optimized for nitrate signaling and utilisation would benefit to both the environment and yield. To answer these questions, the project will take advantage of nitrate-insensitive genotypes that have been found recently, including a knock-out mutant unable to generate reactive oxygen species (ROS) via the RBOHF pathway. That is, we will elaborate on our recent discovery on the essential role of ROS in mediating nitrate signaling in legumes. **Our objectives will thus be to directly assess nitrate capture and metabolic utilization and its potential modulation by ROS using genetic and physiological tools. That way, we will tackle the hypothesis that the down-regulation of ROS-mediated nitrate signaling can be a simple technique to improve nitrate utilization during legume cultivation.**

Methodology

To do so, we will carry out metabolite integrative analyses (metabolomics) and protein (enzyme) analyses (proteomics), to explore the nitrate assimilation pathway in the primary root of the three genotypes (wild-type, nitrate-insensitive *npf6.8* and *rbohf*) grown without or with nitrate, H_2O_2, a ROS, or a H_2O_2 scavenger. Furthermore, we will take advantage of isotopic methods, that is, use ^{15}N-labelled nitrate to trace the fate of assimilated nitrate to main metabolites and measure the nitrogen use efficiency (NUE), which is key parameter for assess the efficacy of plant nutrition. The present project *(i)* aligns with the position of world leader of the Region Pays de la Loire in isotope biochemistry, *(ii)* involves Prof. Guillaume Tcherkez, recently appointed locally as a fellow *Connect Talent*, and *(iii)* utilizes new instruments (IRMS, GC-MS) that come along with his appointment.

Abbreviations used

GC: gaz chromatography ; IR: isotope ratio ; MS: mass spectrometry/spectrometer; NUE: nitrogen use efficiency; ROS: reactive oxygen species

epiDT

Project title: Deciphering the molecular switch of seed desiccation tolerance to improve plant stress tolerance

Acronym: **epiDT**

Project duration: 18 months - Start date: 01/10/2019 End date: 31/03/2021

Key-words: chromatin conformation, epigenetics, desiccation tolerance, seeds, stress

Coordinator: Jérôme Verdier / IRHS – Conserto
Jerome.verdier @inra.fr

PhD student: David WINDELS

Financial support from « Objectif Végétal »: 79,5k€ (Région Pays de la Loire)

Summary:

Drought has challenged food security worldwide, urging the development of drought-tolerant crop varieties. Crops do not withstand severe drought at the vegetative stage, but produce seeds that survive extreme dehydration. The ability to tolerate extreme dehydration **(desiccation tolerance, DT)** is tightly regulated, being switched on during seed maturation and off shortly after germination (DT switch). Seeds can remain alive in the dry state for years and resist extremes of temperature and drought. While major efforts have focused on unravelling the nature of the protective compounds promoting DT, the mechanisms that regulate their expression/accumulation during seed development are poorly understood. In the last few years, new tools have become available enabling the investigation of a yet unexplored level of molecular regulation for DT, the epigenetic landscape.

The **epiDT project aims at unravelling the molecular regulation of the DT switch at the epigenetic level.** Based on preliminary evidence that a specific histone modification tightly represses the regulatory networks responsible for DT in *Arabidopsis thaliana* and *Medicago truncatula* vegetative tissues. We propose combining cutting-edge molecular methods on developing seeds and our capacity to re-induce DT in germinating seeds of the model legume, *Medicago truncatula*, to understand the nature and timing of the DT switch. Then, using the same experimental models, we intend to characterize the minimal core set of genes associated with the DT mechanisms, including regulatory genes and pioneer genes to switch on/off desiccation tolerance in plants.

By unravelling the DT switch activation/repression, our project will target several pivotal agricultural issues, such as **food security and crop adaptation to climate change**, with potential improvement of plant stress tolerance, and conservation of genetic resources, with easier management of short-lived seeds.

THERMIT

Project title: Building subcellular thermometers to study plant mitochondria self-warming in the context of cold tolerance

Acronym: **THERMIT**

Project duration: 18 months - Start date: 01/10/2019 End date: 30/04/2021

Key-words: mitochondria, cold tolerance, freezing tolerance, thermosensor

Coordinator: David Macherel / IRHS – SMS
david.macherel@univ-angers.fr

Financial support from « Objectif Végétal »: 21k€ (Région Pays de la Loire)

Summary:

Context
Plants are considered as ectotherms because their temperature is dictated by their environment. However, the existence of some cases of thermogenic flowers indicates that plant mitochondria have the potential to heat tissues. We demonstrated earlier that isolated pea seed mitochondria were able to perform oxidative phosphorylation at negative temperature, which strongly suggests internal heat generation by the organelles in such freezing conditions. The recent discovery that mitochondria in mammalian cells could reach temperature around 48-50°C, i.e. they are 10°C warmer than their surroundings, reinforces the hypothesis of increased temperature in plant mitochondria. We therefore postulate that mitochondrion self-warming could have an important role in cold tolerance of plants by preserving the bioenergetics functions of the organelle under cold crisis.

Goal:
The goal of the THERMIT project is to construct subcellular thermosensors to indirectly estimate heat production and regulation by mitochondria in plant tissues. The main objective is to prove that plant mitochondria can be warmer than their cellular surroundings, which would contribute to maintain temperature locally, allowing the organelles to maintain a higher level of energy production under cold conditions.

Methodology
We will build genetic constructs encoding a couple of GFP variants that will be used as a ratiometric thermosensor and that will be transiently expressed in the cytosol and in mitochondria of Arabidopsis leaf protoplasts. This will allow to visualize by fluorescence microscopy a temperature difference between the two compartments, in particular at low temperature.

TRANSMISSION

Project title: Transcriptome profiling of plant-bacterial interactions during seed transmission

Acronym: **TRANSMISSION**

Project duration: 24 months - Start date: 01/10/2019 End date: 30/09/2021

Key-words: Seed transmission, seed development, transcriptomes, methylomes, *Phaseolus vulgaris*, *Xanthomonas citri* pv. *fuscans*

Coordinator: Armelle DARRASSE / IRHS – EmerSys
armelle.darrasse@inra.fr

Financial support from « Objectif Végétal »: 21k€ (Région Pays de la Loire)

Summary:

Context
Seeds are colonized by numerous microbial species that could impact plant fitness, especially during the early stages of the plant developmental cycle, such as germination and emergence. To date, the microbial determinants involved in seed transmission and the host response occurring during this stage remain largely unknown.

Goal:
The main goal of the TransMission project is to establish a methodological framework for investigating changes in bacterial transcriptomes and plant transcriptomes/methylomes profiles occurring during seed transmission of bacterial strains.

Methodology
Recent technological developments for isolation of bacterial cells and transcripts within plant tissues will be deployed during the transmission of the plant pathogenic bacteria *Xanthomonas citri* pv. *fuscans* on common bean (*Phaseolus vulgaris*) seeds