First Joint-EFFECTOME-RESISTANCE network meeting

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PROGRAM & ABSTRACT BOOK

From the 12th to the 15th of September 2017, In Banyuls sur Mer, France

With support from the INRA divisions of “Crop health and environment” (SPE INRA), “Forest, Grassland and Freshwater Ecology” (EFPA INRA) and COST action CA16107 “EuroXanth”.


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Effectome/Resistance meeting, September 12-15 2017, Banyuls sur Mer, France

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Tuesday September 12th

14h00-14h15 Welcome and Introductory Remarks by the Organizers

Session 1: Plant receptors in immunity and symbiosis and the genetics of interaction

Chair : Laurent Deslandes

-14h15-14h35: Maud Bernoux (CSIRO, Canberra, Australia) Multiple love in plant immune receptor TIR domains

-14h35-14h55: Stella Cesari (INRA, BGPI-Montpellier, France) Integrated decoy engineering in the rice NLR protein RGA5 enables AVR-PikD recognition in heterologous systems

-14h55-15h15: Elisabeth GRUND (INRA, LIPM-Toulouse, France) Receptor signalling dynamics at the chromatin during transcriptional reprogramming in ETI controlled by RRS1-RPS4 NLR pair

15h15-15h45: Break

-15h50-16h10: Daniel Esmenjaud (INRA, ISA-Sophia, France) Resistance to root-knot-nematodes Meloidogyne spp. in the perennial *Prunus species*

-16h10-16h30: Sébastien Duplessis (INRA, Champenoux, France) Major expansions of immune receptor gene families in the long-lived perennial plant species *Quercus robur* (Oak)

-16h30-17h15: Special Guest from COST-EuroXanth Guido Sessa (Tel-Aviv University, Israel) Manipulation of plant immunity by *Xanthomonas euvesicatoria* type III effectors

17h30-18h30: Poster session

20h00: Dinner

Wednesday September 13th

Session 2: Comparative and population genomics of plant-microbe interactions

Chair : Nemo Peeters

-9h00-9h45 Keynote Lecture: John Jones (James Hutton Institute, Dundee, UK) – Mechanisms underpinning infection of plants by nematodes

-9h45-10h05: Jaime Cubero (Instituto Nacional de Investigación Agraria y Alimentaria, Spain) Pathogenomics of *Xanthomonas arboricola* pv. *pruni*, the causal agent of bacterial spot disease of *Prunus* spp.

-10h05-10h25: Alvaro Perez Quintero (Institut de Biologie de l'École Normale Supérieure, Paris, France) A survey of TAL effector diversity reveals extensive gene conversion as a driver of functional specialization

10h25-10h55: Break

-11h00-11h20: Sylvain Raffaele (INRA, LIPM-Toulouse, France) Adaptation of fungal genomes to multi-host parasitism

-11h20-11h40: Nicolas Frei dit Frey (Toulouse University, France) Identification and characterization of tiny secreted fungal peptides from fungal symbionts and pathogens

-11h40-12h00: Manon Neilen (Utrecht University, Netherlands) Comparative and functional analysis of downy mildew effectors

-12h00-12h20: Jonathan Jacobs (University Louvain-la-Neuve, Belgium) A single cell wall-degrading enzyme defines the basis of Xanthomonas vascular pathogenesis of plants

LUNCH 12h20 – 13h45

-14h00-14h20: Joël F. Pothier (Zürich University of Applied Sciences (ZHAW), Switzerland) Comparative genomic analysis of related virulence genes from two strawberry threats of the bacterial genus *Xanthomonas*

-14h20-14h40: Thierry Marcel (INRA, BIOGER-Versailles-Grignon, France) A genome wide association study to identify genes for pathogenicity of *Zymoseptoria tritici* on bread wheat

Session 3: Strategies for translational research - from knowledge on plant invasion and microbe recognition to sustainable crop production

Chair : Laurence Albar

-14h40-15h25: Keynote Lecture: Guylaine Besnier-Hebert (Biogemma, Chappes, France) Improving *Septoria tritici* blotch resistance in Wheat by Genome Wide Association Studies

15h25-15h55: Break

-16h00-16h20: Laurence Godiard (INRA, LIPM-Toulouse, France) Downy mildew conserved RXLR effectors drive the discovery of sunflower broad-spectrum resistance

-16h20-16h40: Philippe Hugueney (INRA, GAV-Colmar, France) Large-scale metabolomic and genetic analyses in grapevine (*Vitis vinifera*) identify constitutive leaf polyphenols associated to reduced susceptibility to downy mildew

-16h40-17h00: Cyril Brendolise (The New Zealand Institute for Plant & Food Research Limited, New Zeland) Identification and characterization of resistance *to Pseudomonas syringae* pv. *actinidiae* (*Psa*) in kiwifruit – A novel approach

17h30-18h30: Poster session

20h00: Dinner

Thursday September 14th

Session 4: Microbial invasion strategies and host susceptibility – molecular mechanisms, diversity and evolution

Chair : Thomas Kroj

-9h00-9h45 Keynote Lecture: Paul Birch (James Hutton Institute, Dundee, UK) The delivery and activity of late blight effectors

-9h45-10h05: Laila Giordano (INRA, ISA-Sophia, France) Characterization of Individual Domains of the Arabidopsis Receptor-Like Kinase IOS1

-10h05-10h25: Laurent Noel (CNRS, LIPM-Toulouse, France) Immunity at cauliflower hydathodes controls systemic infection by *Xanthomonas campestris* pv. *campestris*

10h25-10h55: Break

-11h00-11h20: Nemo Peeters (INRA, LIPM-Toulouse, France) "EffectorK", a Knowledge-based, curated database for the description and analysis of large scale (effector-plant protein) interactomic data

-11h20-11h40: Rémi Peyraud (INRA, LIPM-Toulouse, France) Plant invasion by necrotrophic fungal pathogens, a systems biology perspective

-11h40-12h00: Andrea Sanchez-Vallet (ETH, Zurich, Switzerland) A highly polymorphic avirulence gene in *Zymoseptoria* induces resistance in wheat

-12h00-12h20: Boris Szurek (IRD, IPME-Montpellier, France) Functional analysis of the TALome of African *Xanthomonas oryzae* pv. *oryzae* reveals a new bacterial leaf blight susceptibility gene candidate

LUNCH 12h20 – 13h45

-14h00-14h20: Albin Teulet (IRD, LSTM-Montpellier, France) When rhizobia develop a symbiosis with legumes thanks their T3SS instead of Nod factors

-14h20-14h40: Joana Cruz (INIAV, Portugal) In vivo transcriptome profiling of *Xanthomonas campestris* pv. *campestris* strains with contrasting virulence

-14h40-15h00: Ganna Reshetnyak (IRD, IPME-Montpellier, France) *Xanthomonas oryzae*-triggered production of atypical rice small RNAs during infection

Session 5: The environment –influence on plant-microbe interactions

Chair : Valérie Geffroy

-15h-15h45 Keynote Lecture: Yuling Bai (Wageningen Universtity, The Netherlands) – Genetics and hormone signalling regulation in tomato response to combined biotic and abiotic stress

15h45-16h10: Break

-16h15-16h35: Nathalie Aoun (INRA, LIPM-Toulouse, France) Quantitative Disease Resistance in the context of global warming: genetic basis of new resistance mechanisms to *Ralstonia solanacearum*

-16h35-16h55: Pasquale Saldarelli (CNR, Istituto per la Protezione Sostenibile delle Piante, Bari, Italy) *Xylella fastidiosa* strain CoDiRO and olive: a transcriptome view of the interactions

Session 6: Signaling – mechanisms, interference by effectors and the physiology of resistance and symbiosis

-16h55-17h40 Keynote Lecture: Jane Parker (Max-Planck-Institute, Cologne, Germany) – Reprogramming the stress response network in effector-triggered immunity

18h15-19h45: Visit of the « cave de l'étoile »

20h30: Gala Dinner

Friday September 15th

Session 6: Signaling – mechanisms, interference by effectors and the physiology of resistance and symbiosis

Chair : Jean-Benoit Morel

-9h00-9h20: Frederik Boernke (Leibniz-Institute of Vegetable and Ornamental Crops, Germany) – Xanthomonas type-III effector proteins targeting host proteasomal protein turnover

-9h20-9h40: Laurent Deslandes (CNRS, LIPM-Toulouse, France) – The *Ralstonia solanacearum* PopP2 effector targets epigenetic readers: a virulence strategy that modulates host epigenome ?

-9h40-10h00: Justin Lee (Leibniz Institut of Plant Biochemistry, Halle, Germany) AvrRpt2-like cysteine proteases suppress activation of specific members of MAMP-activated MAPKs

10h00-10h30: Break

-10h35-10h55: Cyrus Sabbagh (INRA, LIPM-Toulouse, France) Towards a global interactomic map between the *Ralstonia solanacearum* species complex core type III effectors and the tomato proteome

-10h55-11h15: Jianlong Zhao (INRA, ISA-Sophia, France) A MIF like effector of *Meloidogyne incognita* suppresses plant immunity and assists parasitism by interacting with host targets

-11h15-11h45 Concluding remarks

LUNCH 12h00 – 13h00 or lunch box

Posters

Poster n°	Family Name	First Name	Title
1	ALBAR	Laurence	Unraveling the diversity of resistances against Rice yellow mottle virus in African rice
2	ATTARD	Agnes	Two genes, a tubby like protein and a VQ motif-containing protein expressed in Arabidopsis Roots restrict Phytophthora parasitica infection
3	BOCH	Jens	Plant target gene activation by Xanthomonas TALEs
4	CAMBORDE	laurent	Identification and characterisation of a new class of oomycete putative effectors
5	DESAINT	Henri	Identification of resistance mechanisms to Ralstonia and their characterization in Arabidopsis and tomato in a global warming context
6	GADEA	Jose	Characterisation of TAL-effector-mediated resistance to citrus canker using a new variant of Xanthomonas citri.
7	GARCION	Christophe	Deciphering the functions of flavescence dorée phytoplasma effectors
8	GEFFROY	Valerie	Genomic and epigenomic immunity in common bean: the unusual features of NB-LRR gene family
9	GONZALEZ FUENTES	Manuel	Identification and characterization of plant targets of evolutionary-conserved type III effectors from xylem-colonizing bacteria
10	KHAFIF	Mehdi	"Toulouse Plant-Microbe Phenotyping", a new player in the plant-microbe phenotyping business
11	KOEBNIK	Ralf	Comparative genomics identifies a new translocon candidate, HpaT, in type III secretion systems of beta and gamma proteobacteria
12	LANDRY	David	The Study of integrated Decoy in plant NLR proteins
13	LE MARQUER	Morgane	Study of the role of secreted peptides by Rhizophagus irregularis in the establishment of the arbuscular endomycorrhizal symbiosis
14	MOUILLE	Gregory	Understanding of the oligosaccharide production during Botrytis cinerea infection
15	NAGEL	Olivier	The Xanthomonas effector protein XopI suppresses the stomatal immunity of tomato
16	RASTOGI	Meenu Singla	How do plants sense and react to the presence of a bacterial GW effector that targets Arabidopsis AGO1 and induces Effector-Triggered-Immunity?
17	SAUCET	Simon	Understanding how the TIR-NB-LRR-PL resistance gene Ma activates defense in responses to the root-knot nematodes Meloidogyne spp
18	VAN GHELDER	Cyril	TIR-NB-LRR genes in Prunus species: insight into the Post-LRR domain
19	ZARATE CHAVES	Carlos Andrés	Knowledge of Allele Diversity as a Tool to Improve TALE-Targeted Gene Predictions in the Cassava-Xanthomonas Pathosystem
20	ZULUAGA	Paola	Studying the BED protein domain as a new player in plant tolerance to biotic and abiotic stresses

ABSTRACTS FOR ORAL PRESENTATIONS

Multiple love in plant immune receptor TIR domains

Maud Bernoux

CSIRO Agriculture, Canberra, Australia

Plant disease is a major threat to agriculture worldwide, and breeding disease resistance (*R*) genes into crops is currently a key strategy for plant protection. The majority of plant resistance genes encode immune receptors that belong to the NOD-like receptors (NLRs) family. These receptors can recognize specific pathogen effectors, and then activate defense responses. However, the mechanisms controlling NLRs activation and defense signaling are poorly understood. In the flax plant system, the L6 immune receptor is a Toll/interleukin-1 receptor (TIR) domain containing NLR, which confers resistance to the flax rust fungus (*Melampsora lini*) containing the AvrL567 effector. Using a structure-function analysis approach, we previously demonstrated that L6 activation depends on the self-association of its signalling TIR domain. Using similar approaches, we later identified and characterised a different TIR self-association interface in the Arabidopsis paired immune receptor RPS4/RRS1, which is required for the function of this paired receptor but differs from the previously described L6 TIR self-association interface. Recently, the crystal structure of the Arabidopsis SNC1 NLR TIR domain revealed the presence of two self-association interfaces (L6-like, and RPS4-like). Functional studies suggest that both interfaces are required for L6, RPS4 and SNC1 TIR signalling function.

Integrated decoy engineering in the rice NLR protein RGA5 enables AVR-PikD recognition in heterologous systems.

Stella Cesari, Thomas Kroj

INRA UMR BGPI, Campus International de Baillarguet, TA A-54/K, 34398, Montpellier, France

Plant proteins belonging to the superfamily of nucleotide-binding domain and leucine-rich repeat containing proteins (NLRs) are immune sensors which specifically recognize pathogen-derived effectors and induce immune responses. The rice NLR proteins RGA5 and Pikp-1 specifically perceive distinct *Magnaporthe oryzae* effectors by physical binding to their integrated Heavy Metal Associated (HMA) domains. The integrated HMA domains of RGA5 and Pikp-1 share 54% identity and RGA5 recognizes the effectors AVR-Pia and AVR1-CO39 while Pikp-1 enables specific recognition of AVR-PikD. Recently, the crystal structure of the complex formed by AVR-PikD and the HMA domain of Pikp-1 has been reported highlighting residues required for specific HMA- effector binding. By introducing point mutations in the HMA domain of RGA5, we are investigating the possibility of modifying its recognition specificity to enable AVR-PikD-binding and recognition. Using the *Nicotiana benthamiana* heterologous system, we show that an RGA5 variant whose HMA domain has been mutated to 'look like' the HMA domain of Pikp-1 enables recognition of both AVR- Pia and AVR-PikD. Besides, a fragment of RGA5 containing this mutated HMA domain interacts with AVR-Pia, AVR1-CO39 and AVR-PikD in a yeast two-hybrid assay. Stable rice transgenic lines expressing this RGA5 variant are currently being generated and will be assayed for resistance to *M. oryzae* strains carrying the *AVR-PikD*, *AVR1-CO39* or *AVR-Pia* gene. Taken together, these results suggest that targeted modification of integrated decoy domains of NLR proteins is a promising strategy to engineer NLR recognition specificities.

Receptor signaling dynamics at the chromatin during transcriptional reprogramming in ETI controlled by RRS1-RPS4 NLR pair

Elisabeth Grund, Patrick Dabos, Karsten Niefind, Jane Parker, Dominique Tremousaygue, Laurent Deslandes

INRA-CNRS, LIPM, Toulouse, France

The Arabidopsis TIR (Toll-interleukin-1 receptor) – NLR (nucleotide-binding/leucin rich repeat receptors) pair RRS1-R/RPS4 confers resistance to the acetyltransferase effector PopP2 of *Ralstonia solanacearum*. PopP2 acetylates a key lysine within the C-terminal WRKY DNA-binding domain of RRS1-R, causing disruption of RRS1-R DNA association and the dislodging of the RRS1-R/RPS4 complex from chromatin sites, eventually resulting in the activation of RPS4-dependent immune response (Le Roux et al., 2015; Sarris et al., 2015). Previous studies showed that RRS1-R/RPS4 host cell reprogramming depends on the immunity regulator EDS1 (Griebel et al., 2014). EDS1 is a lipase-like protein that as shown to be essential for immunity mediated by TIR NLRs (Heidrich et al., 2011) and is also necessary for basal resistance (Zhou et al., 1998). To date, the mechanisms by which NLRs activate immunity pathways are still poorly understood. This project focuses on the study of receptor signaling dynamics at the chromatin during transcriptional reprogramming in ETI controlled by RRS1-R/RPS4. We specifically aim to elucidate at which chromatin sites pre-activated RRS1-R complexes bind and if the effector–activated RRS1-R relocates on the DNA. Furthermore we will investigate if the chromatin binding of pre- and post-activated RRS1-R depends on RPS4 or EDS1. Additionally we will compare chromatin-binding sites of pre- and post-activated RRS1-R with the binding sites of RRS1-S, a natural variant of RRS1-R from the Col-0 ecotype. Contrary to RRS1-R, RRS1-S does not confer resistance to PopP2. Nonetheless, PopP2 acetylates RRS1-S at the same lysine within the WRKY domain, causing DNA disassociation (Le Roux et al., 2015). To monitor TNL genome-wide associations to chromatin sites and to quantify transcriptional outputs we use DNA adenine methylation IDentification (DamID) coupled to Illumina DNA sequencing (Dam-Seq), Chromatin Immuno-Precipitation and RNA-Seq.

Resistance to root-knot-nematodes *Meloidogyne* spp. in the perennial *Prunus* species

Van Ghelder C.[1], Saucet S.[1], Duval H.[2], and Esmenjaud D.[1]

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[2]INRA, UGAFL, Groupe porte-greffe, F-84143 Montfavet, France

Prunus spp. are severely damaged by the root-knot nematodes (RKNs) *Meloidogyne* spp. and resistant rootstocks are a promising alternative to nematicides. A sustainable resistance is needed in this durable plant-nematode interaction and current breeding aims at pyramiding resistance (*R*) genes in interspecific *Prunus* rootstocks. *R* genes with different RKN spectra have been characterized and mapped in plums (*Ma* and *Rjap*), almond (*RMja*) and peach (*RMia*). *Ma* triggers a hypersensitive response and confers a complete-spectrum and heat-stable resistance to RKNs, which makes it a promising *R* gene for pyramiding strategy. Sustainability of *Ma* resistance has been successfully challenged by *M. incognita* under controlled conditions in multi-year experiments. *Prunus Ma R* plants remained resistant after co-cultivation with RKN-infested susceptible tomato plants, whereas *Mi-1 R* tomato plants did not. An experiment of natural infestations by the three major RKN species, *M. incognita, M. javanica* and *M. arenaria*, is also in progress in Morocco in order to challenge *Ma* resistance under field conditions. *Ma* is a TIR-NB-LRR encoding gene extended by a huge C-terminal post-LRR (PL) region comprising five repeated exons. Because this unique PL structure has only been detected in *Prunus* species, its putative link with the wide-spectrum resistance of *Ma* will be studied with chimeric constructs relying on TNL domain shifts between the *Ma* resistant and susceptible alleles. Recent data suggest that the *RMja R* gene in almond, conferring resistance to *M. javanica* but not to *M. incognita*, is the orthologue of *Ma*. This gives new opportunities to characterize the molecular determinants responsible for the RKN resistance spectrum in *Prunus* species.

Major expansions of immune receptor gene families in the long-lived perennial plant species *Quercus robur* (Oak)

Sébastien Duplessis

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Oaks are important trees of many forest ecosystems and landscapes and they provide important services to human societies. Their longevity is part of their emblematic cultural and historical importance. Such long-lived sessile species must persist in the face of a wide range of abiotic and biotic threats over their lifespans. The sequencing, assembly and annotation of the genome of the oak *Quercus robur* allow investigation of genomic features required to achieve such a long lifespan. Interestingly, the gene families encoding immune receptors such as NB-LRRs and LRR-RLKs related families show major expansions in this tree genome, accounting for almost 10% of the whole gene complement compared to the 1-2% usually recorded for these two gene categories in other plant species. Based on reconstructed paleohistory and survey of orthologous gene groups in 14 selected genomes of tree and herbaceous species, we show that the arsenals of genes involved in the perception and response to pathogens and parasites are significantly over-represented in the oak genome and in perennial species. After introducing the oak genome project and basic genomics features, the detailed annotation and expansions of NB-LRR genes will be presented.

This paper is part of the ANR project GENOAK, led by Christophe Plomion at INRA Pierroton and done in collaboration with the CEA-Genoscope Centre National de Séquençage, in the frame of a large national consortium.

https://arachne.pierroton.inra.fr/QuercusPortal/index.php?p=OAK_GENOME_SEQUENCING

Manipulation of plant immunity by *Xanthomonas* type III secreted effectors

Georgy Popov, Doron Teper, Anil Madhusoodana Girija, Bharat Bhusan Majhi, Guy Sobol and Guido Sessa

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Many plant pathogenic bacteria utilize the type III secretion system to deliver effector proteins into host cells, interfere with host cellular functions, and promote disease. A main function of type III effectors is to disarm plant defense responses by manipulating signaling components of plant immunity. *Xanthomonas euvesicatoria* (*Xe*) strains, which cause bacterial spot disease in tomato and pepper plants, deliver a pool of about 35 type III effectors into plant cells to contribute to pathogenesis. We utilized a combination of functional screens, biochemical and genetic approaches to identify *Xe* type III effectors that manipulate plant immunity and uncover their biochemical properties, mode of action and plant targets. By a comprehensive screen carried out in Arabidopsis protoplasts, we discovered that more than 50% of the *Xe* effector pool interferes with changes in gene expression and defense responses associated with pattern-triggered immunity (PTI). Further analysis of XopAE, a PTI-suppressing effector, revealed that this effector is expressed in an operon and encodes an E3 ubiquitin ligase. We also demonstrated that certain *Xe* effectors interfere with effector-triggered immunity (ETI). For example, XopQ inhibits ETI by interacting with a tomato scaffold protein of the 14-3-3 protein family that is required for plant immunity. Conversely, XopAU, which encodes a catalytically active protein kinase, activates plant defense responses by interacting and activating the immunity-associated MAP kinase kinase MKK2. Significance and models depicting molecular strategies used by *Xe* effectors to manipulate plant immunity will be discussed.

Mechanisms underpinning infection of plants by potato cyst nematodes

John T. Jones[1,2], Sebastian Eves-van den Akker[1,3], James Price[1,2], Sophie Mantelin[1], Kerry Leslie[1,2] Peter Thorpe[1] & Terry K. Smith[2].

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Cyst nematodes, including the potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* are remarkable plant pathogens. The survival stage of the nematode is able to remain dormant in the soil for periods of up to 20 years until a susceptible host is detected growing nearby. This synchronisation of the life cycle of the nematode and its host is achieved by the unhatched nematode entering a stage of diapause which is broken by the presence of root diffusates that stimulate hatch of the juvenile from the egg. We have analysed the lipidome and proteome of nematode eggshells in order to try to determine the molecular mechanisms underpinning this process. A recent study (Manosalva *et al.* Nature Communications 6: 7795) has identified a group of glycolipid ascarosides as nematode PAMPs that are recognised by diverse plants to elicit defence responses. Our analysis shows that ascarosides are a key component of the nematode eggshell, explaining the importance of these compounds to nematode biology. Analysis of eggshell associated proteins has revealed the presence of various enzymes and ligand binding proteins that allow us to propose a model for the control of hatching.

Following infection of the host, the nematode produces a large multinucleate syncytium on which it depends for the food required to develop to the adult stage and protects this structure from host defence responses for several weeks. These processes are mediated by effectors produced in the pharyngeal gland cells of the nematode and secreted into the plant. We have identified several hundred effectors from PCN using a variety of approaches, including through identification of a promoter element associated with genes expressed in the gland cells (Eves-van den Akker *et al.,* 2016; Genome Biology 17:124). We have subsequently demonstrated that the same approach can be used to identify a different promoter element associated with gland cell sequences in unrelated nematodes and that this can be used to identify new effectors from these species. Given a sufficiently robust training set, promoter identification may therefore represent a means for identifying comprehensive effector lists from species for which no protein signature is associated with effectors. In our current work we have identified a “core” list of effectors present in all nematodes that produce syncytia for which genome or transcriptome information is available. Functional studies on some of these sequences have identified proteins that suppress host defence responses.

Pathogenomics of *Xanthomonas arboricola* pv. *pruni*, the causal agent of bacterial spot disease of *Prunus* spp.

Jerson Garita-Cambronero and Jaime Cubero

Departamento de Protección Vegetal, Laboratorio de Bacteriología, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria (INIA), Madrid, Spain.

Xanthomonas arboricola pv. *pruni* (*Xap*) is a subinfraspecific group of plant pathogenic bacteria within the *X. arboricola* species. Xap is considered a quarantine pathogen in the European Union because it is responsible for bacterial spot of stone fruits and almond. The expansion of this pathogen in Europe has strengthened the efforts to understand virulence factors associated with this pathosystem. In our study, Genome sequencing of *Prunus*-virulent and non-virulent *Xanthomonas arboricola* strains, isolated during Spanish outbreaks of this disease, unveiled that non-virulent strains were not comprised in the Xap subinfraspecific group. In addition, those potential virulence factors associated to bacterial spot disease were revealed after genomic comparative analysis between these two groups of strains. For the analysis a database, with more than 400 genes described in *Xanthomonas* as important during the different stages of the disease process, was created and used for searching those homologous genes only present in the virulent strains.

As a result, variations in essential genes involved for in the bacterial population establishment, such those of methyl accepting chemotaxis proteins, TonB-dependent transporters or the sensors of the two-component regulatory system, were elucidated. Beside this, variations in the profile of frimbrial and non-fimbrial adhesins were also shown. In addition, a wide variation in those components associated with cell-wall degrading enzymes as well as with the Type III secretion system and its related effectors was also revealed. This study has pointed out the way for future functional studies that will allow to understand the *Xap-Prunus* interactions as well as the development of specific disease control strategies.

This work was supported financially by the Instituto Nacional de Investigación y tecnología Agraria y Alimentaria (INIA) project RTA2014-00018-C02-01

A survey of TAL effector diversity reveals extensive gene conversion as a driver of functional specialization

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TAL effectors (aka TALEs) are repeat containing proteins found in the genus of phytopathogenic bacteria *Xanthomonas*. They are able to bind DNA through their repeat region and induce genes in the host plant. Thus variation in repeat number and order can lead to diversification of virulence functions and evasion of recognition by the host. Variation in TAL effector repeats and repeat sequence between different taxonomic groups has remained largely unexplored. In this study we describe the diversity found in a large set (957) of TAL effector sequences from 19 *Xanthomonas* pathovars and 3 groups of organisms containing TALE-like sequences, and we use this information to infer evolutionary mechanisms for TAL effectors. For this, we analyzed variation at three levels of organization in TAL effectors sequences: *1)* the individual repeat level, *2)* the TAL effector sequence level and *3)* the repeat "motifs" level. The results of these analyses showed loss of repeat sequence diversity through the *Xanthomonas* genus suggesting some degree of concerted evolution. The "homogenization" of repeat sequences seems to favor high rates of duplication and recombination between repeats as evidenced by patterns of repeat substitution and insertion/deletions in TAL effector sequences. To asses the extent of recombination, particularly gene conversion, between TAL effectors, we designed the program RecTAL to identify shared motifs of repeats. This program identified multiple motifs in our dataset, leading us to propose that the swapping of repeat blocks between TAL effectors is a motor for TAL effector specialization that allows for fast functional diversification through the acquisition of new targets in the host plants.

Adaptation of fungal genomes to multi-host parasitism

Malick Mbengue[1], Thomas Badet[1], Remi Peyraud[1], Olivier Navaud[1], Marielle Barascud[1], Stefan Kusch[1], Mark Derbyshire[2], Richard P. Oliver[2], Adelin Barbacci[1], Sylvain Raffaele[1]

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The range of hosts that parasites can infect is a key determinant of the emergence and spread of disease. In fungal pathogens, host range varies from a single host genotype (specialists) to hundreds of unrelated species (generalists). Current theories often assume that pathogens specialize mostly on one host, and how broad host range fungi adapt to the diverse species they can infect remains elusive. We first generated a finished genome assembly for the plant pathogenic fungus *Sclerotinia sclerotiorum*, notorious for its broad host range encompassing several hundreds of species. We next analyzed polymorphisms at the genome level in field isolates of *S. sclerotiorum*, revealing patterns of purifying selection. Biased patterns of synonymous substitutions underpinned increased codon optimization in *S. sclerotiorum* but not a specialist fungal pathogen. Virulence genes were consistently enriched in highly codon-optimized genes of generalist but not specialist species. We next scanned the genome of ~50 fungi belonging all major clades, and found that codon optimization correlates with host range across the fungal kingdom. We conclude that codon optimization is related to the capacity of parasites to colonize multiple hosts. To test whether *S. sclerotiorum* uses specific strategies to infect diverse plants, we analyzed global fungal gene expression during the colonization of *Arabidopsis thaliana*, tomato and sunflower as representative of major dicot clades infected by this fungus. We found core and host-specific gene sets. The putative function of host-specific regulated genes suggests that they could contribute to fungal adaptation to specific plant lineages. Together, our analyses demonstrate that broad host range parasitism in S. sclerotiorum involved both universal and multiple specialized genome adaptations.

Identification and characterization of tiny secreted fungal peptides from fungal symbionts and pathogens.

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Some recent studies have shown that proteins secreted by arbuscular mycorrhizal fungi may be important regulators of the arbuscular mycorrhizal symbiosis (Kloppholz *et al*., 2011, Tsuzuki *et al*., 2016, Kamel *et al*. 2017). We recently identified *R. irregularis* transcripts coding for putative secreted proteins that are commonly upregulated in the roots of different hosts: medicago, brachypodium and the liverwort Lunularia (Kamel *et al*., 2017). Some of these proteins display a structure that resembles ascomycete sexual pheromone precursors. These proteins are known to be processed into small peptides that are secreted to initiate a cellular reprogramming, leading to cell fusion of opposite mating types. In the case of *R. irregularis*, only clonal reproduction has yet been described, but recent genomic data questions this asexual behavior (Ropars *et al*., 2016). These peptides may therefore have acquired new functions during evolution. Recent data also demonstrate that some of these peptides undergo cyclization and act as toxins (Nagano *et al*., 2016, Ding *et al*., 2016). In *R. irregularis*, the size of the predicted peptides ranges from three to fifty amino acids. The development of a bioinformatic pipeline applied to a large set of fungal genomes led us to observe that glomeromycota are not the only one with an important diversity of proteins containing such peptides. They are also present in ascomyceta as expected, but also in other early diverging fungi and in basidiomycota. Functional characterization of a selection of *R. irregularis* pheromone-like peptides is currently underway. We have also investigated the role of a peptide encoded by *Botrytis cinerea*. Exogenous application of this peptide dramatically enhances the virulence of this pathogenic fungus on common bean and arabidopsis. Overall, with the help of our comparative genome analysis and our current functional approaches, we will extrapolate on the functions of these widespread tiny secreted peptides.

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Comparative and functional analysis of downy mildew effectors

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Peronospora farinosa (Pfs) is an obligate biotrophic oomycete that causes downy mildew in spinach. In terms of crop loss, it is the most important disease in the spinach cultivation industry. Upon infection, downy mildews secrete effector proteins that either function inside or outside the host cell. Effectors manipulate plant cell processes and can suppress defence responses of the plant. The aim of my project is to identify effector proteins that are conserved in downy mildew species/oomycetes and to study their role in the infection process. A reference genome and transcriptome of Pfs were generated and used to predict the secretome of Pfs1. In a comparative approach using OrthoMCL, the secreted proteins of Pfs were compared to the predicted secretomes of eight other plant pathogenic oomycetes (downy mildew and Phytophthora species). From the OrthoMCL clusters, conserved proteins that have known effector domains, like RXLR and crinkler motifs, were selected for further functional studies. Conserved effector proteins are likely to be of crucial importance to the infection strategy of downy mildews/oomycetes and are, therefore, an appealing target for resistance breeding. Moreover, this approach allows for the identification of unique effectors that are species- or genus-specific and that have evolved more recently, possibly to adapt to specific hosts. The virulence function of selected Pfs effectors is now being studied by identifying interacting host proteins using the yeast two hybrid (Y2H) system. Interaction screens of Pfs effectors with an oligo-dT-primed Arabidopsis cDNA Y2H library revealed candidate target proteins. Among the interaction candidates are NAC transcription factors and other proteins that have a potential link to plant defense. The interactions between effectors and candidate targets will be validated in planta and their possible role in plant resistance or susceptibility will be further functionally analyzed. Studying the function of conserved effectors and their interacting plant proteins will lead to a better understanding of the molecular interactions between plant and pathogen that may be conserved between plant species.

A single cell wall-degrading enzyme defines the basis of Xanthomonas vascular pathogenesis of plants

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Microbial pathogens cause vascular and non-vascular diseases of plants and animals. Vascular diseases are particularly destructive because the pathogen moves systemically through the host vasculature such as the water-transporting xylem. Non-systemic pathogens are equally important to farmers but remain restricted to the non-vascular tissue near the site of infection. The basis of vascular and non-vascular pathogenesis is unknown. Here we describe the role of cell wall degradation in the evolution and biology in the phytobacterial genus *Xanthomonas*. *Xanthomonas* comprises diverse vascular and non-vascular pathogens of over 200 plant species. We show that a single, vascular pathogen-unique cell wall-degrading enzyme called CelA contributes to systemic pathogenesis in multiple *Xanthomonas* species. We determined that CelA was conserved only in systemic pathogenic bacteria in the genera *Xanthomonas, Xylella* and *Ralstonia* but absent in non-systemic Gram-negative plant-pathogenic bacteria. Notably expression of this cell wall-degrading enzyme in two distinct non-vascular pathogen species, barley-infecting *Xanthomonas translucens* and rice-infecting *Xanthomonas oryzae*, permitted systemic pathogenesis of their respective host plants. Genomic analysis revealed that non-systemic *Xanthomonas* pathogens appear to have inactivated this trait upon adapting to the non-vascular plant environment suggesting that they derived from related vascular subgroups. Overall this work provides a framework to describe pathogen emergence based on symptom development and tissue specificity in an important pathogen genus.

Comparative genomic analysis of related virulence genes from two strawberry threats of the bacterial genus *Xanthomonas*

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Strawberry plants are grown in all temperate regions of the world and their fruit production is of high economic importance. *Xanthomonas fragariae* was long considered the only bacterial pathogen on strawberry causing a yield reduction and an economic loss. The bacterium causes angular leaf spots, which are water soaked spots leading to plant tissue necrosis. Virulence-related gene content was reported in a draft genome of *X. fragariae*, including a type IV secretion system and a type III secretion system effector repertoire containing multiple effectors, including several putative new ones.

In 1993, a new disease called 'bacterial leaf blight' was observed on strawberry plants in northern Italy and a new pathogen, *Xanthomonas arboricola* pv. *fragariae*, was reported as the causal agent. However this bacterium has an unclear virulence status on strawberry as no new disease report was made recently and symptom observation was ambiguous during artificial inoculations.

The sequenced genomes of both species allow for gene screening potentially related to virulence. If slight gene content variability was observed within *X. fragariae* strains, most of the genes showed significant differences between the two species, possibly explaining the lack of virulence of *X. arboricola* pv. *fragariae* strains.

Distinct groups of *X. fragariae* strains were highlighted based on molecular markers, suggesting that bacterial origin could be multiple and that this genetic variability could confer different virulence effects. An inoculation test was performed with *X. arboricola* pv. *fragariae* and strains belonging to different groups of *X. fragariae*. Even though variation of virulence was observed, it did not follow the obtained strain grouping of *X. fragariae.* Finally symptoms could not be observed for *X. arboricola* pv. *fragariae*.

A genome wide association study to identify genes for pathogenicity of *Zymoseptoria tritici* on bread wheat

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Zymoseptoria tritici is a fungal pathogen of wheat responsible for the septoria leaf blotch disease. This disease is a major concern in bread and durum wheats growing areas worldwide. It can be controlled by fungicide treatments and the use of resistant wheat cultivars. To date, 21 resistance genes and 89 QTLs for resistance to septoria leaf blotch have been mapped in bread wheat. Despite this large availability of resistant sources, it remains a challenge to improve the resistance levels of wheat elite cultivars because of the mostly quantitative nature of wheat-septoria interactions and of the very high level of genetic diversity within pathogen populations. Our objective is to understand the genetic architecture of pathogenicity in *Z. tritici* by identifying its determinants and revealing the specificity of their interaction with resistance determinants in wheat. We have undertaken a genome wide association mapping approach (GWAS) to identify pathogenicity genes in *Z. tritici*. A collection of more than 2000 isolates has been established in France, from which 109 isolates have been selected for resequencing and pathogenicity assays. In so doing, we obtained the virulence spectra of the 109 French isolates, revealing the efficiency of known resistance *STB* genes in France, and allowing to detect known and unknown *STB* genes present in French elite cultivars. Furthermore, combining the precision of our pathology assays, the selection of wheat genotypes well characterized for their resistance determinants, and the precision of GWAS in our *Z. tritici* population, we have identified several candidate avirulence genes interacting with known resistance genes in bread wheat. Thus far, the small secreted protein *AvrStb6* is the first avirulence gene to be identified and functionally validated in this important wheat pathogen.

Improving *Septoria tritici* blotch resistance in Wheat by Genome Wide Association Studies

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Septoria tritici leaf blotch (STB), caused by the ascomycete *Mycosphaerella graminicola* (anamorph *Septoria tritici*), is an economically important disease of wheat. Breeding for resistance to STB is the most effective means to control the disease and can be facilitated through the use of molecular markers. The main goals of the study are to rank genotypes according to their resistance to STB and identify markers associated with a better resistance. It aims to support breeders to select and develop varieties able to answer farmers' demand in the future.

BreedWheat project (BW) has developed a field trials network using a panel of 220 European elite lines over 3 years in which 4 experiments in field were conducted by French breeding companies and one experiment was done by BIOGER on seedlings. Phenotypic measurements at 3 different dates after inoculation allowed us to classify genotypes regarding to STB resistance. Using the TaBW420k Axiom array develop within the BreedWheat project, a Genome Wide Association Study (GWAS) was carried out using 250k polymorphic markers.

1807 marker-trait associations (MTA) were detected in at least two different experiments representing 58 LD blocks. Moreover, some MTA found on seedling colocalize with Stb6 gene. It appears that using the most associated marker in the block, we can be predictive at 98 % for the presence/absence of the stb6 resistant allele in BW varieties. In addition, among the 58 LD blocks associated, five were selected for SNP densification and confidence interval reduction.

In fine, we developed a set of markers associated with STB resistance. These markers will provide a useful tool for breeders to perform a selection of varieties with a better resistance to STB in the future.

Acknowledgments: All the BreedWheat partners (ANR-10-BTBR-0003) involved in field trials and Etienne Paux *et al.* for the genotyping data on the 220 varieties using TaBW420k Axiom Array.

Downy mildew conserved RXLR effectors drive the discovery of sunflower broad-spectrum resistance

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Plasmopara halstedii is an obligate biotroph oomycete causing downy mildew disease on sunflower, *Helianthus annuus*, an economically important oil crop. *P. halstedii* pathotypes are defined by their divergent virulence profiles in a set of sunflower differential hosts carrying different *Pl* resistance genes, not yet cloned. During the last decades *P. halstedii* pathotypes showing new virulent patterns appeared, concomitantly with the breakdown of *Pl* resistance loci used in fields. Finding broad-spectrum and more durable resistance against downy mildew disease is therefore an agronomic issue. We focused on *P. halstedii* RXLR effectors conserved among pathotypes to drive the discovery of sunflower broad range resistance. High-throughput sequencing and *in silico* methods led us to identify potential RXLR effectors, 58% of which were conserved at the protein level in 17 *P. halstedii* pathotypes. Thirty of them expressed in pathogen infecting leaves were cloned for transient expression experiments. Seventeen cloned effectors suppressed Pathogen Triggered Immunity induced by *Phytophthora infestans* INFESTIN1 in *N. benthamiana,* suggesting they were true effectors. Subcellular localization of the effectors was performed in sunflower cells and their ability to trigger hypersensitive responses in sunflower lines was tested. Four cloned RXLR effectors including C1 induced HR in resistant lines carrying different *Pl* loci. Testing the C1 effector on F3 populations segregating for resistance indicated co-segregation of C1 induced-cell death activity with the *Pl* resistance locus. This *Pl* locus was finely mapped in a region of 3 Mbp of the sunflower genome thanks to AXIOM SNP arrays.

Large-scale metabolomic and genetic analyses in grapevine (*Vitis vinifera*) identify constitutive leaf polyphenols associated to reduced susceptibility to downy mildew

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Downy mildew, due to the Oomycete *Plasmopara viticola*, is one of the major leaf diseases of grapevine (*Vitis vinifera*). Although cultivated grapevines do not possess resistance genes against *P. viticola*, susceptibility to downy mildew varies significantly among varieties. Indeed, some varieties are highly susceptible, while others are more tolerant to this disease. In order to understand the bases of these variations, we used a 120---genotypes grapevine population to performed non---targeted metabolomic and genetic analyses, coupled to high---precision phenotyping for susceptibility to downy mildew. These analyzes revealed that constitutive accumulation of specific polyphenols in leaves correlated with better tolerance to downy mildew. The protective role of these polyphenols was then validated in a core collection of 256 grape varieties representing the genetic diversity of *Vitis vinifera*. This protective role proved to be much superior to that of stilbenes, which have long been associated to defense against downy mildew. Future work will determine whether such polyphenol---based constitutive metabolic defenses can efficiently complement resistance genes in grapevine breeding programs, for enhanced durability of future downy mildew---resistant varieties.

Identification and characterization of resistance to *Pseudomonas syringae* pv. *actinidiae* (Psa) in kiwifruit – A novel approach

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The virulent pathovar of *Pseudomonas syringae pv. actinidiae* (Psa, biovar 3) is the causal agent of the recent outbreak of bacterial canker disease in kiwifruit which is responsible for severe economic losses worldwide. In an effort to provide new resistance/tolerance markers to our kiwifruit breeding programme and accelerate the development of resistant cultivar, a new approach was developed to identify potential resistance genes to Psa effectors across other plant species. Recent study of the Psa repertoire of effectors has revealed that biovar 3 of Psa contains specific effectors and effectors broadly represented in other *P. syringae* species [1]. We have identified a subset of these effectors that are recognized in the plant model *Nicotiana benthamiana*. Taking advantage of the availability of the *N. benthamiana* genome (Boyce Thompson Institute for Plant Research), we developed a new approach to identify the resistance gene(s) required for the recognition of these effectors based on genome wide identification and systematic silencing of all the potential *N. benthamiana* R genes. Here we present a proof-of-concept experiment in which we confirmed using our R gene RNAi library that the Pto/avrPto-triggered HR is cancelled when silencing the Prf R gene as previously described [2]. We also demonstrate using other known avirulence factors that this approach can potentially be applied to identify R genes responsible for the recognition of any effectors/proteins originating from a broad range of plant pathogens that trigger an HR in *N. benthamiana*. We will present the preliminary results of the library screen in response to some of the Psa effectors which identified new R genes involved in Psa effector recognition.

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The delivery and activity of late blight effectors

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The oomycete *Phytophthora infestans* causes late blight, globally the most serious disease of potato. *P. infestans*, during its interaction with potato and other hosts, such as the model solanaceous plant *Nicotiana benthamiana*, produces finger-like protrusions called haustoria, which form intimate interactions with host plant cells. We show that haustoria are the sites of secretion of both apoplastic effectors, which act outside of host cells, and cytoplasmic effectors, which are delivered inside living plant cells to suppress defences and manipulate other host processes. We show that these effectors are delivered by different secretion pathways. Much effort has been focussed on the roles of cytoplasmic RXLR effectors: identifying their target proteins in the host and characterising their roles in manipulating those host proteins to facilitate disease development. This presentation will reveal the latest progress we have made in studying host target proteins of RXLR effectors, in particular focussing on so-called susceptibility (S) factors that are used by the pathogen to negatively regulate immunity.

Characterization of Individual Domains of the Arabidopsis Receptor-Like Kinase IOS1

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Oomycetes are devastating filamentous pathogens that impact ecosystems and agriculture. Our research aims at characterizing the molecular mechanisms that govern the establishment of disease in host plants. To sense the environment, plant cells possess more than 200 plasma membrane-spanning receptors, which are composed of extracellular leucine-rich repeats (LRRs) and an intracellular kinase domain. We previously identified the Arabidopsis receptor-like kinase "Impaired Oomycete Susceptibility 1" (IOS1), which downregulates abscisic acid (ABA) signaling and contributes to the infection success of filamentous, biotrophic pathogens such as oomycetes and the powdery mildew fungus (Hok *et al.*, 2011; Hok *et al.*, 2014). The extracellular region of IOS1 is composed of LRRs and a domain, which shares similarities with malectin from animals. Animal malectins bind carbohydrates and participate in monitoring proper folding of glycoproteins in the endoplasmic reticulum (ER). We observed retention of IOS1 in the ER, which appears to be mediated through the malectin-like (ML) domain. Yeast two-hybrid screens with the extracellular IOS1 domain identified an ER-localized protein. I will present and discuss results from this analysis.

Immunity at cauliflower hydathodes controls systemic infection by *Xanthomonas campestris* pv. *campestris*

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Hydathodes are water pores found on leaves of a wide range of vascular plants and are the sites of guttation. We report here on the detailed anatomy of cauliflower and Arabidopsis hydathodes. Hydathode surface presents pores resembling stomata giving access to large cavities. Beneath, the epithem is composed of a lacunar and highly vascularized parenchyma offering a direct connection between leaf surface and xylem vessels. Arabidopsis hydathode pores were responsive to ABA and light similar to stomata. The flg22 flagellin peptide, a well-characterized elicitor of plant basal immunity, did not induce closure of hydathode pores in contrast to stomata. Because hydathodes are natural infection routes for several pathogens, we investigated hydathode infection by the adapted vascular phytopathogenic bacterium *Xanthomonas campestris* pv. *campestris* (*Xcc*), the causal agent of black rot disease of *Brassicaceae*. Microscopic observations of hydathodes six days post inoculation indicated a digestion of the epithem cells and a high bacterial multiplication. Post-invasive immunity was shown to limit pathogen growth in the epithem and is actively suppressed by the type III secretion system and its effector proteins. Altogether, these results give a detailed anatomic description of *Brassicaceae* hydathodes and highlight the efficient use of this tissue as an initial niche for subsequent vascular systemic dissemination of *Xcc* in distant plant tissues.

“EffectorK”, a Knowledge-based, curated database for the description and analysis of large scale (effector-plant protein) interactomic data

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I will present our collective work in generating, displaying and characterizing the large-scale interactomic data, mainly (but not only) based on yeast-two-hybrid data. Our four labs have team-up to share the multiple screens for plant protein interacting with pathogen effectors. The biological objects are the effectors of the plant pathogenic bacteria *Ralstonia solanacearum, Xanthomonas campestris* and the root knot nematode *Meloidogyne incognita*, the screened plants are mainly Tomato and Arabidopsis. Homology links enable to group homologous effectors and homologous plant targets, with a link to other model plants. Recent large scale interactomic curated data (1, 2) was also included. The main objective of this database is to provide a shared working environment for the discovery of potential meaningful interactions (for instance plant hubs interacting with several effectors). As the data included grows, a layer of network analysis will be required to filter the information. EffectorK was set up by the LIPM-bioinformatics team and enables to host curated relations between objects of different nature. In a near future, plant gene expression under specific pathogenic interactions will also be added.

Plant invasion by necrotrophic fungal pathogens, a systems biology perspective

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Plant-pathogens interactions are highly complex systems in biology. Outcome of the interaction relies on an intricate and dynamic virulence-resistance network. Among the molecular components of this network are the profusion of pathogen's effectors dedicated to subvert plant environment, the battery of plant immune systems perceiving and countering the pathogens invasion, and the metabolic capacities of the pathogens to benefit from the hijacked plant resources. Systems biology may offer an effective approach to understand the functioning of this intricate network and predict the outcome of such complex interactions. In the coming years, predictive capacities may represent novel opportunities to understand pathogens emergence and evolution, as well as designing robust resistance strategies in crops. We reconstructed the genome-scale model of the broad-host range necrotrophic fungal plant pathogen *Sclerotinia sclerotiorum*. The mathematical model encompasses the metabolic network of the fungi (1075 reactions), a macromolecule module describing the secretion of effectors into the plant environment (201 reactions), and a plant cell wall degradation module linking the plant components with the activities of the secreted plant cell wall degrading enzymes (247 reactions). First, we conducted simulations using the model to reveal the extent of the plant cell wall of *Arabisdopsis thaliana* degraded by the fungi and involved in physical barrier breakdown and/or nutrition. Our analysis revealed a specialization of the nitrogen nutrition capacities of *S. sclerotiorum* toward conserved structural motifs within the plant cell wall proteins. Secondly, we used the model to infer the invasion program of *S. sclerotinorum* upon *A. thaliana* infection by integrating the Omics data (RNAseq) we collected on hyphae progressing through the plant tissues ("the front line") and the center of the necrosis ("the back line"). Our analysis revealed cooperation at multicellular level via division of labor between "the front line", which produces the virulence factors and faces the defense of the plant, and the center of the colony dedicated to resources storage and translocation to support "the front line".

A highly polymorphic avirulence gene in *Zymoseptoria* induces resistance in wheat

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Zymoseptoria tritici is a globally distributed pathogen that colonizes wheat plants, causing devastating damages. The fungus has a peculiar infection cycle including a long asymptomatic phase followed by a necrotrophic phase that coincides with an increase in fungal biomass. Several resistance proteins from specific host cultivars control the development of particular avirulent isolates. These resistance proteins are thought to recognize specific avirulence factors. Until now, only one avirulence gene has successfully been cloned. By means of genetic mapping, we have identified a new avirulence gene. The gene is upregulated upon infection and encodes a cysteine-rich small secreted protein, which indicates that it has a role during infection. Disruption of this gene in the avirulent isolate led to an increase in virulence. Complementation experiments showed that polymorphism in the coding sequence is responsible for the difference in virulence between the two isolates. The avirulence gene is present in all of 131 investigated isolates from across the world and exhibits high sequence polymorphism. The genomic region surrounding the gene has low conserved synteny and only in the virulent isolate two insertions rich in transposable elements are present. Remarkably, in vitro expression of a selection marker under a constitutive promoter is substantially lower when inserted at the position of the avirulence gene compared to a random position in the genome. These data indicate that this region is silenced when the pathogen is not in contact with the plant and that the transposable elements might regulate the expression of the gene. Highly controlled gene expression regulation, high sequence polymorphism and localization in a highly dynamic genomic environment highlight a major role of this avirulence gene in plant colonization.

Functional Analysis of The TALome of African *Xanthomonas oryzae* pv. *oryzae* Reveals a New Bacterial Leaf Blight Susceptibility Gene Candidate

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Most *Xanthomonas* species translocate Transcription Activator-Like (TAL) effectors into plant cells to function like specific plant transcription factors via a novel programmable DNA-binding domain. Rice-pathogenic *Xanthomonas oryzae* pv. oryzae (*Xoo*) strains contain multiple TAL effector genes in their genome. While one or two act as major virulence factors, the relative contribution of each of the other members to *Xoo* pathogenicity remains unclear. To address that question, the TAL effector repertoires, herein referred to as TALomes, of three African *Xoo* strains have been first analyzed using whole-genome single molecule, real-time sequencing. A phylogenetic analysis of the three TALomes combined with *in silico* predictions of TAL effector targets showed that African *Xoo* TALomes are highly conserved, genetically distant from Asian ones, and closely related to TAL effectors from the bacterial leaf streak pathogen *Xanthomonas oryzae* pv. oryzicola (*Xoc*). More precisely, 9 clusters of TAL effectors could be identified among the three TALomes, with 6 clusters showing no more than 2 polymorphic repeat variable diresidues (RVDs) and 3 clusters showing higher level of variations in their RVDs. To address their function, 9 *TAL* effector genes from the Malian *Xoo* strain MAI1 and 4 allelic variants from the Burkinabe *Xoo* strain BAI3, thus representing most of the TAL effector diversity in African *Xoo* strains, were expressed in the *TAL* effector-deficient *X. oryzae* strain X11-5A for systematic gain-of-function assays. Inoculation of the susceptible rice variety Azucena lead to the discovery of 3 TAL effectors promoting higher virulence to X11-5A, including two TAL effectors previously reported to target the susceptibility (*S*) gene *OsSWEET14* and the novel major virulence TAL effector TalB. Our most recent data on the functional analysis of this new major virulence TAL effector and its targets will be presented.

When rhizobia develop a symbiosis with legumes thanks their T3SS instead of Nod factors

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Some non-photosynthetic bradyrhizobia strains possess the unusual ability to nodulate some legume plants in the absence of Nod factors, such as *Bradyrhizobium elkanii* USDA61 strain on soybean or *Bradyrhizobium* ORS3257 on *Aeschynomene indica.* In these last cases, a functional type III secretion system (T3SS) is required, suggesting that T3SS effectors, also called Nops for Nodulation Outer Proteins, injected in the host cell can directly activate the nodulation signaling pathway by bypassing the Nod factors perception. An *in silico* analysis, combining a TblastN of known Nops and the search of the *tts-box* motif corresponding to the DNA binding site of the T3SS transcriptional regulator TtsI, revealed 39 putatives *nop* genes on the ORS3257 genome. To identify the *nop* genes involved in the nodulation process, two mutagenesis strategies have been used covering 29 out of the 39 identified *nops* genes : i) the deletion of regions containing several clustered effectors ii) insertional inactivation of *nop* genes found isolated. After inoculation on *A. indica*, three of these mutations show important effects ranging from the induction of uninfected nodules to the drastic reduction in the nodules number. In the latter case, the inactivation of a single gene corresponding to a new putative effector is responsible of the phenotype observed. Taken together, these data indicate this new symbiotic process relies on a cocktail of symbiotic effectors that should play distinct role in the infection and nodule organogenesis processes.

In vivo transcriptome profiling of *Xanthomonas campestris* pv. *campestris* strains with contrasting virulence

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Black rot disease caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*) is the most important bacterial disease of Brassicas worldwide. Although several genomic approaches have been used to characterize the molecular mechanisms of host-pathogen interaction, little is known about *Xcc* regulation of virulence during pathogenesis. Using a RNA-Seq approach, the *in vivo* transcriptome profiling of two contrasting virulence *Xcc* strains – L-vir (low-virulent strain) and H-vir (high-virulence strain) – inoculated on two cultivars of *B. oleracea* (cv. 'Wirosa' and cv. 'Bonanza') was carried out, yielding a total of 530M 69bp reads. The established transcriptome of *Xcc* strains was not dependent on the host tested, suggesting that virulence is an inherent characteristic of the pathogen. In contrasting virulence strains, a total of 154 differentially expressed genes (DEGs) were identified. The most represented functional categories were 'pathogenicity and adaptation' (14%), followed by 'signal transduction and regulation' (9%) and 'transport' (9%). Among DEGs, Type III effector coding genes *xopE2* and *xopD* were induced in L-vir strain, while *xopAC*, *xopX* and *xopR* were induced in H-vir strain. While *xopE2* was the least expressed DEG in L-vir strain, XCC3695, coding for a poorly characterized oxidoreductase, was the most expressed DEG in H-vir strain. Overall, low virulence appears to be the combined result of impaired sensory mechanisms, reduced detoxification of reactive oxygen species, decreased motility, higher production of pathogen- associated molecular patterns (PAMPs), associated to an overexpression of avirulence proteins and a repression of virulence proteins targeting the hosts' PAMP-triggered immune responses.

Xanthomonas oryzae-triggered production of atypical rice small RNAs during infection

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Eukaryotic regulatory small RNAs (sRNA) range from 20 to 24 nucleotides and are ubiquitously present in plants. As mediators of transcriptional and post-transcriptional gene silencing, they play an important role in regulating growth, development and stress responses. In the plant cell, sRNAs are mostly endogenous but can also derive from viral sequences or originate from the surrounding environment, symbionts or pathogens. sRNA biogenesis begins with the cleavage of double-stranded RNA precursors by one of the dicer-like enzymes (DCL). The resulting sRNA duplexes are then protected from degradation by HEN1-mediated methylation, and the mature guide sRNA strand is incorporated into the RNA-induced silencing complex (RISC). RISC targets complementary nucleotides to dampen gene expression. Early insight on sRNAs in plants revealed their role in antiviral defense and they are now extensively studied in response to diverse pathogens. However most of these functional data comes from *Arabidopsis* and other systems remain unexplored. Here we describe a novel class of sRNA in rice (*Oryza sativa*) associated with foliar diseases caused by *Xanthomonas oryzae*. Analysis of our high-throughput sRNA sequencing data suggests that *Xanthomonas*-induced small RNAs (xisRNAs) possess features of regulatory sRNA and may target genes involved in plant immune signaling or sRNA production. xisRNAs biogenesis is still enigmatic but we showed that it depends on some canonical sRNA pathways components. Moreover, transcription of associated protein-coding loci is required for xisRNAs accumulation. Our results further indicate that *X. oryzae* uses its virulence arsenal of type III effectors for xisRNA production. Finally, we will report on our effort to address the significance and genuine silencing targets of these xisRNA during disease.

Genetics and hormone signaling regulation in tomato response to combined biotic and abiotic stress

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Crop losses due to abiotic and biotic stresses are important threats to achieving the goal of feeding the world population with fewer inputs on less arable lands. Each of the stress factors impacting food production is subject of many researches, but only few studies address the response to multiple stresses at the same time. Aiming to unravel the requirements for resilience of the tomato crop against the combination of biotic and abiotic stresses, we started in 2011 to explore tomato and powdery mildew interaction under salt stress conditions. We uncovered specific responses under salt stress and powdery mildew combination which were dependent on stress intensity and disease resistance mechanisms. Hormones, with their complex regulation and cross-talk, were shown to play a key role in the combined stresses. Few WRKY transcription factor family were identified regulating powdery mildew resistance and cell death in a salt stress dependent manner. In my talk, I will assemble our gained insights in genetics and hormone signaling regulation in tomato response to combined biotic and abiotic stress. Understanding of plant resilience to stress combinations can lead towards the goal of breeding crops with a good performance under diverse environmental conditions.

Quantitative Disease Resistance in the context of global warming: genetic basis of new resistance mechanisms to *Ralstonia solanacearum*

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In the context of climate warming, plants will be facing an increased risk of epidemics as well as the emergence of new highly aggressive pathogen species. Although a permanent increase of temperature strongly affects plant immunity, the underlying molecular mechanisms involved are still poorly characterized. In this study, we aimed to uncover the genetic bases of resistance mechanisms remaining efficient at elevated temperature to the *Ralstonia solanacearum* species complex (RSSC), one of the most harmful phytobacteria causing bacterial wilt. To identify quantitative trait loci (QTLs) associated with natural variation of response to *R. solanacearum*, we adopted a genome wide association (GWA) approach using worldwide and local natural accessions of *Arabidopsis thaliana*. In the worldwide population, one major QTL of resistance was identified at 30°C in the early stages of infection. We have functionally validated, with a reverse genetic approach, the involvement of a strictosidine synthase-like 4 (SSL4) protein that shares structural similarities with animal proteins known to play a role in animal immunity. Several other QTLs were identified in the local population of Arabidopsis and the underlying genes are still under functional characterization.

Xylella fastidiosa strain CoDiRO and olive: a transcriptome view of the interactions

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A strain of *Xylella fastidiosa* is ravaging the olive trees of Southern Apulia, Italy (Saponari et al., 2013). The bacterium causes the Olive Quick Decline Syndrome (OQDS), a disease showing symptoms of apical and marginal leaf scorching, extensive branch and twig dieback and death of the trees (Martelli et al., 2016). Transmission tests demonstrated that the *Xylella fastidiosa*-olive infecting strain is transmitted by the xylem-feeding meadow froghopper *Philaenus spumarius* (Saponari et al., 2014) whereas genome studies proved that it belongs to the subspecies pauca, sequence type 53 (Giampetruzzi et al., 2015). Field observations allowed to identify a possible source of resistance in the olive cv Leccino, which reacts with milder symptoms, and hosts a much lower bacterial concentration of bacterial cells than the locally grown cv Ogliarola salentina. A global transcriptome profiling revealed that a higher number of genes is altered upon *Xylella fastidiosa* infection in the susceptible cv Ogliarola salentina compared to the resistant cv Leccino, with respect to the healthy plants of the same cultivars (Giampetruzzi et al., 2016). Gene expression analysis showed that both cultivars perceive the presence of the bacterium with the involvement of membrane signaling receptors, whose characterization is ongoing. Analysis of altered genes of the susceptible cv Ogliarola salentina (expansin, early-late inducible proteins, late embryogenesis abundant proteins and involvement of the abscissic acid pathway) indicated that plants are subjected to a intense water stress. A further transcriptome analysis of the recently discovered *Xylella*-resistant olive cv FS17® is in process. Results of these studies will be presented.

Reprogramming the stress response network in effector-triggered immunity

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We're studying how plants regulate and execute resistance downstream of NLR receptors activated by pathogen effectors inside host cells. All studied TNL (TIR-NLR) immune receptors in Arabidopsis and other plant species signal via EDS1 nucleocytoplasmic complexes. To identify and position induced anti-microbial pathways within the plant stress network, we have been examining EDS1-controlled transcriptional reprogramming triggered by the Arabidopsis RRS1-RPS4 TNL receptor pair recognizing bacterial effector, AvrRps4. I will report on an Arabidopsis genetic suppressor screen and, from it, the discovery of a TNL immunity sector which protects SA-mediated defenses against pathogen or genetic interference. This ETI signaling mechanism employs EDS1/PAD4 inside nuclei to inhibit the transcriptional activity of a JA-response master regulator, MYC2, thereby dampening SA-antagonizing JA pathways and boosting SA resistance which provides a crucial barrier against biotrophic pathogens. This resistance sector accounts for ~ 1/3 of the RRS1-RPS4 immune response and we're applying a combination of sector genetic depletion, RNA-seq and structure-guided EDS1/PAD4 mutations to unpick the remaining resistance pathways in ETI. Our findings in Arabidopsis serve as a useful reference point to explore whether the same or different stress network properties apply in a crop species.

Xanthomonas type-III effector proteins targeting host proteasomal protein turnover

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Xanthomonas campestris pv. *vesicatoria* (Xcv) is the causal agent of bacterial spot disease on pepper and tomato plants. To overcome the basal defence of plants, Xcv translocates about 30 type III effector proteins (T3E) via its type III secretion system into the host cell. These T3Es are able to manipulate host cellular processes involving vesicle trafficking, the ubiquitin/proteasome system (UPS) and gene expression, although the host cellular target remains unknown for many of them. Evidence is emerging that manipulation of the UPS might be an effective and widespread virulence strategy of bacterial invaders to promote pathogenesis. We have identified Xcv T3Es that interfere with host proteasomal turnover either globally or specifically through different mechanisms. XopJ, a T3E from the widespread YopJ-family of effector proteins, acts as a protease to degrade the 26S proteasome subunit RPT6. This leads to an inhibition of proteasomal protein degradation and a subsequent attenuation of salicylic-acid mediated defence responses. We could show that XopJ interferes with the degradation of NPR1 through the proteasome and thus providing a mechanical explanation for XopJ virulence function. Another example of a T3E interfering with host proteasomal turnover is provided by XopS. This T3E interacts inside the plant cell nucleus with a protein pair consisting of the transcription factor WRKY40 and an E3-ubiquitin ligase to prevent proteasomal degradation of WRKY40. Stabilization of WRKY40 subsequently interferes with the induction of defence related gene expression and attenuates symptom development. Possible biochemical mechanisms of XopS action will be discussed.

The *Ralstonia solanacearum* PopP2 effector targets epigenetic readers: a virulence strategy that modulates host epigenome?

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Arabidopsis intracellular immune receptors RRS1-R (Resistance to Ralstonia solanacearum 1) and RPS4 (Resistance to Pseudomonas syringae 4) function as a dual TNL (Toll/Interleukin1-Nucleotide Binding-Leucine rich repeat) resistance complex localized on DNA and able to recognize, among other effectors, Ralstonia solanacearum PopP2. RRS1-R contains a C-terminal WRKY DNA-binding motif, characteristic of the zinc-finger class of WKRY plant transcription factors, that is targeted by Popp2 acetyltransferase activity. RRS1-R acetylation by PopP2 dislodges the RPS4/RRS1-R complex from DNA and leads to the activation of plant immunity. PopP2 inhibits basal resistance by acetylating many other defensive WRKY transcription factors and therefore contributes to bacterial virulence in plants lacking the resistance complex. For a better understanding of the virulence functions of PopP2, we screened a Y2H cDNA library to identify additional targets of PopP2. Among the interacting partners identified, we isolated 2 Arabidopsis bromodomain-containing proteins. The bromodomain (BRD), known to recognize acetyl-lysine residues on proteins, is the conserved structural module in chromatin-associated proteins. Although BRD proteins are epigenetic readers that had been shown to play a crucial role in the development of several diseases including cancer, inflammation and viral replication in human, almost nothing is known about their role in plants. Our data demonstrate that (i) these 2 BRD proteins physically associate with PopP2 within the nucleus of living plant cells and, (ii) their BRD is modified by PopP2 enzymatic activity. Using Histone peptide arrays, we show that these BRDs are able to bind specifically to the acetylated N-terminal tail of a particular histone. Our data suggest that PopP2 uses an original mechanism that targets chromatin-related processes, probably aimed at the regulation of host gene transcription to favor pathogen invasion. We also developed a Dam-ID based approach in order to identify the chromatin environment targeted by PopP2 activities. Together, this study should provide important insights on a virulence strategy deployed by a Type III effector to manipulate host epigenome.

AvrRpt2-like cysteine proteases suppress activation of specific members of MAMP-activated MAPKs

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To enable infection, pathogens deploy effector proteins to subvert host cellular signaling elements. Mitogen-activated protein kinase (MAPK) cascades are key signaling pathways in eukaryotic immunity. We show that the well-studied bacterial effector, AvrRpt2, blocks MAMP-induced activation of Arabidopsis MPK4/MPK11 but not the MPK3/MPK6 pathway. This suppressive effect is dependent on the protease activity of AvrRpt2 but it does not involve direct degradation of MPK4/MPK11. By comparing MPK4/MPK11-suppression activity of numerous putative AvrRpt2 homologs from pathogenic and non-pathogenic bacteria, we could exclude the role of RIN4, a well-known target of AvrRpt2. This means that other AvrRpt2-cleaved product(s) is/are responsible, which may include members from the RIN4/NOI family. We propose that this selective manipulation of subsets of MAPK activation may be a novel virulence function to presumably fine-tune defense signaling. AvrRpt2 also appears to affect more immune signaling steps in plants than previously known.

Towards a global interactomic map between the *Ralstonia solanacearum* species complex core type III effectors and the tomato proteome

Cyrus Raja Rubenstein Sabbagh[1], *Alice Morel*[1], *Fabien Lonjon*[1], *Patrick Barberis*[1], *Stéphane Genin*[1], *Fabienne Vailleau*[1,2] *and Nemo Peeters*[1]

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The *Ralstonia solanacearum* species complex (RSSC) virulence strategy relies mainly on numerous type III effectors (T3Es) injected inside the plant cells *via* a type III secretion system (T3SS). Thanks to the currently known genomic sequences of the different plant pathogenic strains of the RSSC, our group was able to identify a list of 30 conserved or "core" T3Es [1]. As these core-T3Es have been conserved through the evolution of the highly diverse RSSC, with strains affecting different host plants, we hypothesize that they must be important for virulence. Each core T3E gene was cloned from the *R. solanacearum* GMI1000 strain and further sub-cloned into two (Prey and Bait) yeast-2-hybrid (Y2H) plasmids. One of our main objectives is to identify putative tomato targets for all of these 30 core-T3Es. This is currently carried out by a systematic Y2H screening against a tomato root cDNA library. A set of 15 different T3Es have been screened up to now. These screenings allowed the identification of numerous candidate tomato targets, including different signaling proteins (RLKs), transcription factors and enzymes. Interestingly, various "hubs" (candidate tomato targets putatively interacting with more than one T3E) were also identified. In order to better characterize some potential hubs, we are currently testing all the tomato targets identified against all the 30 core T3Es using the Y2H-pairwise approach. Several candidate genes were selected to be silenced by VIGS in order to test their contribution to the bacterial wilt disease establishment in tomato. Our aim is to both better understand the mechanisms of disease development and provide means for future bacterial wilt control strategies.

A MIF like effector of *Meloidogyne incognita* suppresses plant immunity and assists parasitism by interacting with host targets

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The macrophage migration inhibitory factor (MIF) has extensive biological functions including modulates innate and adaptive immune responses. Previous evidence showed that animal parasites secreted MIF like proteins in evading host immune response during infection, which provides us inspiration to study the MIF in plant parasitic root-knot nematodes (RKN). In our research, we identified four MIF isoforms (MiMIFs) in *Meloidogyne incognita*. One of the four MiMIFs, MiBMIF has the tautomerase activity and the ability to protect nematode from harmful H_2O_2. Overexpressing MiBMIF in Arabidopsis or *in planta* RNA interference causes 30% increase or 60% reduction in the number of galls and nematodes, respectively. Moreover, overexpression of MiBMIF in plants suppressed the deposition of callose, producing amount of H_2O_2 in plant root and the expression of marker genes and MAPK cascade by flg22-triggered plant immunity. In addition, $[Ca^{2+}]_{cyt}$ influx was impaired in overexpressing MiBMIF Arabidopsis. Immunoprecipitation (IP) of MIF-interacting proteins followed by Co-IP and BiFC assays revealed that MiBMIF specifically interacted with two Arabidopsis proteins mainly involved in stress responses and signal transductions. Arabidopsis lines overexpressing these genes showed lower susceptibility to *M. incognita*, whereas T-DNA mutants exhibited an increase susceptibility to nematode. Our results provide the first known functional evidence that *M. incognita* utilizes MiMIFs to suppress host immune responses and promote parasitism.

ABSTRACTS

FOR POSTER PRESENTATIONS

Poster1: Laurence Albar

Unraveling the diversity of resistances against *Rice yellow mottle virus* in African rice

Hélène Pidon, Christine Tranchant-Dubreuil, François Sabot, Sophie Chéron, Harold Chrestin, Alain Ghesquière, Laurence Albar

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The *Rice yellow mottle virus* (RYMV) is a Sobemovirus that represents one of the major threat for rice cultivation in Africa. The dissection of natural resistance based on genetic and genomic approaches revealed distinct high resistance pathways, associated with at least three different genes. *RYMV1* and *RYMV2* resistance genes encode respectively a susceptibility factor, whose interaction with a viral protein is required for the virus cycle (Albar *et al.,* Plant J., 2006), and a putative nucleoporin involving in the regulation of defense mechanisms (Orjuela *et al.*, MPMI, 2013). We recently mapped the first dominant resistance gene against RYMV, *RYMV3*, in a 15 kb interval containing a CC-NBS-LRR gene, that is currently under validation (Pidon et al., TAG, 2017). The study of high resistance pinpointed the interest of the African cultivated species of rice (*Oryza glaberrima*) as resistance source, compared to the Asian cultivated species (*O. sativa*), worlwide cultivated. Diversity of resistance genes was investigated in a collection of 300 *O. glaberrima* accessions. The 41 highly resistant accessions identified carry a known or a candidate resistance alleles on at least one resistance gene and 10 different candidate resistance alleles were identified. This analysis revealed a surprisingly high variability in *RYMV1* and *RYMV2* genes in the least genetically diverse crop grass known, and traces of selection on these genes will be investigated.

Poster2: Agnes Attard

Two genes, a tubby like protein and a VQ motif-containing protein expressed in Arabidopsis Roots restrict *Phytophthora parasitica* infection

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Little is known about the responses of plant roots to filamentous pathogens, particularly for oomycetes. We investigated the overall changes in gene expression in *A. thaliana* roots challenged with *P. parasitica*. We analyzed various infection stages, from penetration by the pathogen and establishment of the interaction to the switch from biotrophy to necrotrophy. Among the genes modulated during the 10 first hours after infection, we identified 2 genes. One gene encoding a tubby like protein, *TLP* was found down regulated during biotrophy. Another gene encoding a VQ motif-containing protein, *VQ* is locally upregulated in plant roots, early in infection. Inactivation of both genes significantly perturbed susceptibility to *P. parasitica* infection. The two corresponding proteins contribute to root defense response, restricting the growth of the oomycete pathogen. We also showed that the defense associated to TLP and VQ was independent of known defense pathways associated to aerial parts of plants. Moreover, *TLP* expression is modulated during the onset of infection, nevertheless inactivation of TLP perturbs only late stages of infection. Our data suggest that the particular genetic program specifically activated during penetration may determine the outcome of pathogen invasion. These results and the functional analysis of these genes will be presented.

Poster3: Jens Boch

Plant target gene activation by *Xanthomonas* TALEs

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Transcription activator-like effectors (TALEs) are bacterial proteins that operate as transcription factors in eukaryotic cells. Rice-pathogenic *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) and *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) use large numbers of TALEs to manipulate host plant cells and cause bacterial leaf blight and bacterial leaf streak, respectively, which are devastating disease of rice. We analyzed the TALE gene repertoire of *Xoo* strains from various locations to gain insights into the evolution of TALE-based pathogenicity. To compare TALEs between strains we grouped TALEs from *Xoo* and *Xoc* into classes according to their DNA-binding specificities and developed a unified nomenclature. Comparison of TALEs within classes and at genomic locations revealed different mechanisms that shape TALE evolution. TALE proteins bind DNA via 34-amino acid tandem repeats with each repeat recognizing one base in the target DNA. In addition to being versatile virulence factors, their modular architecture allows rearrangement of TALE repeats to generate artificial transcription factors with any tailored DNA-binding specificity. This feature enables a systematic approach to study gene activation in plants. We assembled a large collection of TALEs targeting a well-known virulence target *SWEET* promoter in rice to scan from which positions within a promoter TALEs can induce gene expression. Our analysis shows that TALEs mediate gene activation from a variety of positions with significantly varying efficiencies. Our data further suggest that TALEs require additional promoter elements to induce transcription. Surprisingly, TALEs also induced gene expression when positioned in reverse orientation. This observation expands the possibilities for identifying new virulence targets. However, reverse-binding TALEs show a different positional requirement which suggests some mechanistic differences in comparison to forward-binding TALEs.

Understanding gene activation by TALEs will enable a refined search for virulence targets.

Poster4: Laurent Camborde

Identification and characterisation of a new class of oomycete putative effectors

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Among the oomycete lineage, the *Aphanomyces* genus includes plant and animal pathogenic species. By comparative genetic analyses, we found that the legumes pathogen *A. euteiches* genome is characterized by a large repertoire of small secreted protein coding genes (SSP), highly induced during plant infection, and not detected in other oomycetes. Functional analyses of several AeSSPs revealed that AeSSP1256, which contains a nuclear localisation signal suggesting its translocation to the nuclei of host cells, increases plant susceptibility to infection. Yeast two hybrid screening permits identification of few *Medicago truncatula* proteins predicted to be nuclear localized and involved in various nucleic acids processes. Molecular experiments are in progress to decipher the effect of AeSSP1256 in these interactions and the role of this new effector on plant infection strategy.

Poster5: Henri Desaint

Identification of resistance mechanisms to *Ralstonia solanacearum* and their characterization in Arabidopsis and tomato in a global warming context

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Scenarios related to climate warming, widely accepted, predict the expansion as well as the emergence of new pathogens in new areas. They also foresee an increase in the occurrence of epidemics crops will have to face. In addition, recent studies reported that a permanent elevation in temperature (3-7°C) generally inhibits the main known resistance mechanisms to pathogens. In these conditions, significant yield losses are expected, threatening the maintenance of global food security. Strikingly, the mechanisms involved in these inhibitions are still poorly understood. Therefore, identifying and studying the genetic and molecular basis of defense mechanisms allowing plants to cope with epidemics under higher temperature condition is critical. The *Ralstonia solanacearum* species complex (RSSC) causes tremendous yield losses worldwide, in more than 200 plant species, especially in crops of the *Solanaceaous* family such as tomato and potato. Despite the identification of resistance QTLs to RSSC in different species, and the use of tolerant cultivated crops, studies reporting their underlying genetic basis have been only well characterized in *Arabidopsis.* Moreover, fluctuations in environmental parameters, such as a moderate and permanent increase in temperature, affect host resistance to *R. solanacearum* in several plant species as we have also recently shown in *Arabidopsis.* Consequently, a Genome Wide Association (GWA) mapping approach was developed in the team to explore natural genetic variation in a worldwide collection of *Arabidopsis* in response to *R. solanacearum* and to uncover new sources of resistance to RSSC that remain efficient under elevated temperature conditions. Several Quantitative Trait Loci (QTLs) depending on strains and inoculation procedures were fined map. These results will be presented as well as my PhD project that aims at 1) functionally validating the most promising candidate genes underlying these QTL and characterizing the conferred tolerance in *Arabidopsis* and tomato and 2) explore the genetic variability of wild-type tomato accessions in response to RSSC in elevated temperature conditions.

Poster6: José Gadea

Characterisation of TAL-effector-mediated resistance to citrus canker using a new variant of *Xanthomonas citri*.

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Xanthomonas citri subspecie citri (X. citri) is the causal agent of Asiatic citrus canker. We have previously identified a natural variant, X. citri AT, that triggers a host-specific defense response in Citrus limon and C. sinensis. In this work, this defense response is assessed by transcriptomic, physiological and ultrastructural analyses. X. citri AT triggers in resistant plants a hypersensitive response (HR-like) associated with the interference on biofilm development and arrest of bacterial growth. This response involves an extensive transcriptional re-programming setting in motion cell wall reinforcement, oxidative burst and accumulation of salicylic acid and secondary metabolites. Ultrastructural analyses revealed subcellular changes involving the activation of autophagy-associated vacuolar processes. Furthermore, this defense response protects plants from disease upon subsequent challenges by pathogenic Xanthomonas. The X. citri AT bacterial gene causing the deployment of HR-like responses in the plant appears to be a short (7.5 repeats) variant of the pathogenic pthA4 TAL-effector. The mode of action of this short TAL is also mediating transcriptional activation of plant genes, as mutated version of the TAL effector in nuclear localization or activation domains hampers the triggering of the HR-like response. DNA-binding assays using double-stranded microarrays indicate that this short TAL-effector is able to bind DNA. Identification of plant targets involved in the triggering of this canker resistance phenotype is underway. This knowledge will help to rationally exploit the plant immune system as a biotechnological approach to manage citrus canker, and reveals biological functionality for short TAL-effectors.

Poster7: Christophe Garcion

Deciphering the functions of “flavescence dorée” phytoplasma effectors

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The flavescence dorée disease of grapevine is an epidemic disease which represents a threat for French and European vineyards where the insect vector *Scaphoideus titanus* is settled. Management efforts can maintain the disease at a low level but with economic and environmental costs. The flavescence dorée agent is a phytoplasma, a wall-less bacterium that is currently not cultivated *in vitro*. With the aim to better understand the interaction between the phytoplasma and its host plant, we have undertaken a study on flavescence dorée phytoplasma effectors. Bioinformatics analyses have led us to build a short list of thirteen candidate effectors. In order to get a glimpse of their mode of action during the infection, we determined their subcellular localization in plant cells. However, as these effectors may as well be directed against targets from the insect vector, a comparative study of their expression level and subcellular localization has been initiated between insect and plant cells. Efforts are also focused on the determination of the effector targets. In the future, the identification of effector plant targets may help to establish strategies to generate grapevine varieties more resistant to the flavescence dorée disease.

Poster8: Valérie Geffroy

Genomic and epigenomic immunity in common bean: the unusual features of NB-LRR gene family

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In plants, a key class of genes comprising most of disease resistance (R) genes encodes Nucleotide-binding leucine-rich repeat (NL) proteins. Access to common bean (*Phaseolus vulgaris*) genome sequence provides unparalleled insight into the organization and evolution of this large multigene family (~400 NL) in this important crop. As observed in other plant species, most common bean NL are organized in complex cluster of genes with the particularity to be located in subtelomeric regions close to terminal knobs containing the satellite DNA *khipu*. Phylogenetically related NL are spread between different chromosome ends, suggesting frequent exchanges between non-homologous chromosomes. NL peculiar location, in proximity to heterochromatic regions, led us to study their DNA methylation status using a whole-genome cytosine methylation map. In common bean, NL genes displayed an unusual body methylation pattern since half of them are methylated in the three contexts, reminiscent of the DNA methylation pattern of repeated sequences. Moreover 90 NL were also abundantly targeted by 24nt siRNA, with 90% corresponding to methylated NL genes. This suggests the existence of a transcriptional gene silencing mechanism of NL through the RdDM (RNA-directed DNA methylation) pathway in common bean which has not been described in other plant species.

Poster9: Manuel Gonzalez Fuente

Identification and characterization of plant targets of evolutionary-conserved type III effectors from xylem-colonizing bacteria

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Xanthomonas campestris pv. *campestris* (*Xcc*), the causal agent of black rot disease on *Brassicaceae*, and *Ralstonia solanacearum* (*Rs*), the causal agent of bacterial wilt on more than 200 plant species, are both xylem-colonizing bacteria whose pathogenicity relies on the injection of type III effectors (T3Es) in the plant cells. Despite their differences in host range, infection strategy and effectome composition, these two evolutionary distant bacteria share 6 orthologous T3Es (6 in *Xcc* and 9 in *Rs*) likely targeting orthologous processes in the host. Our project aims to identify biologically relevant interactors of those shared T3Es focusing on *Arabidopsis thaliana*, a common host of both pathogens. To this end, high throughput yeast-two hybrid screenings are conducted to detect the plant targets that interact with orthologous T3Es from both pathogens. Simultaneously, physiological and transcriptional analyses on transgenic arabidopsis lines expressing single T3Es will be performed to identify interactors and pathways involved in the plant response. Finally, pathogenicity of multiple T3E mutants in *Xcc* and *Rs* will be evaluated. The biological relevance of identified interactions will be further tested and characterized through reverse genetics approaches. Altogether, this project will provide a better understanding of the biological role of these T3Es conserved through evolution providing new resources for breeding crops with enhanced tolerance to these two important bacteria and possibly to other vascular pathogens.

Poster10: Mehdi Khafif

"Toulouse Plant-Microbe Phenotyping", a new player in the plant-microbe phenotyping business

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The Toulouse Plant-Microbe Phenotyping platform or "TPMP" is a state-of-the-art automated platform capable of acquiring image data (different spectra) of small to large plants under biotic and/or abiotic stress in two experimental setups: A "phenopsis", dedicated to small plants (up to 500 plants), and 2 automated greenhouses, with a total capacity of 350 large plants (in 3l pots). Automated image acquisitions (top view for the small plants; top and side view for the larger plants) can be performed with visual, UV and Near-infra red spectra, enabling the estimation of plant growth and development (leaf area, biomass estimation), fitness of the plant (photosynthesis efficiency measurement, relative water status), but also the tracking of microbe colonization and plant symptoms (GFP signal, auto-fluorescence of diseased leaves). The facility will be certified to be able to host quarantine organism. The environmental condition can be modulated in terms of temperature, hygrometry, light intensity (Phenopsis and Greenhouse) and CO2 content (Phenopsis) enabling the study of plant biotic interactions under changing environments.

The TPMP phenotyping infrastructure is open for service as of now and we hope that this presentation will inspire people from the effectome-resistance community to come and work with us.

Poster11: Ralf Koebnik

Comparative genomics identifies a new translocon candidate, HpaT, in type III secretion systems of beta and gamma proteobacteria

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Xanthomonas translucens is the causal agent of bacterial leaf streak, the most common bacterial disease of wheat and barley. To cause disease, most xanthomonads depend on a highly conserved type III secretion system, which translocates type III effectors into host plant cells. Mutagenesis of the conserved type III secretion gene *hrcT* confirmed that the *X. translucens* type III secretion system is required to cause disease on the host plant barley and to trigger a non-host hypersensitive response in pepper leaves. Type III effectors are delivered to the host cell by a surface appendage, the hollow Hrp pilus, and a translocon protein complex that inserts into the plant cell plasma membrane. Homologs of the *Xanthomonas* HrpF protein, including PopF from *Ralstonia solanacearum* and NolX from rhizobia, are thought to act as a translocon protein. Comparative genomics revealed that *X. translucens* strains harbor a noncanonical *hrp* gene cluster, which rather shares features with type III secretion systems from *Ralstonia solanacearum*, *Paraburkholderia andropogonis*, *Collimonas fungivorans* and *Uliginosibacterium gangwonense* than other *Xanthomonas* spp. Surprisingly, none of these bacteria, except *R. solanacearum*, encode a homolog of the HrpF translocon. Here, we aimed at identifying a candidate translocon from *X. translucens*. Notably, genomes from strains that lacked *hrpF*/*popF*/*nolX* instead encode another gene, called *hpaT*, adjacent to and co-regulated with the type III secretion system gene cluster. An insertional mutant in the *X. translucens hpaT* gene, which is the first gene of a two-gene operon, *hpaT-hpaH*, was non-pathogenic on barley and did not cause the hypersensitive response or programmed cell death in non-host pepper similar to the *hrcT* mutant. The *hpaT* mutant phenotypes were partially complemented by either *hpaT* or the downstream gene, *hpaH*, which has been described as a facilitator of translocation in *Xanthomonas oryzae*. These findings suggest that both HpaT and HpaH may contribute to the injection of type III effectors into plant cells.

Poster12: David LANDRY

The Study of integrated Decoy in plant NLR proteins

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Abstract:

Plant defense against pathogens often involve NLR proteins that can recognize specific effectors. Most of these plant NLR proteins have three conserved domains: Nucleotide-Binding domain (NB-ARC), Toll-Interleukin Receptor domain (TIR) or Coiled-Coil domain (CC), and Leucine Rich Repeat domain (LRR). However, recent findings showed that NLR proteins could also have an additional non-conserved protein domain that could play the role of a decoy[1]. These decoys mimic an operational host target. They can be used as a "trap" by the plant to detect pathogen effectors, leading to activation of plant immunity. For instance, RRS1, a NLR protein from *Arabidopsis thaliana* has a WRKY decoy domain. This WRKY domain can be acetylated by the effector PopP2 from *Ralstonia solanacearum*[2]. This modification triggers plant immunity showing the central role of decoys in some defense responses. Sarris and colleagues[3] analyzed proteomic data of several plants species to identify NLR proteins with putative decoys. We used these data to clone several putative decoy domains, at first from *Arabidopsis thaliana* and *Solanum lycopersicum*. The main objective of this project is to create a library of decoys and provide a resource available for the scientific community. In addition, by a Yeast-Two-Hybrid experiment, we checked the interaction of the first cloned decoys with effectors conserved in the *Ralstonia solanacearum* species complex. Preliminary data seems to confirm that at least one tomato decoy could interact with two different *Ralstonia solanacearum* effectors.

Keywords: Decoys, Effectors, NLR protein, Yeast-Two-Hybrid, *Ralstonia solanaceraum*.

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Poster13: Morgane Le Marquer

Study of the role of secreted peptides by *Rhizophagus irregularis* in the establishment of the arbuscular endomycorrhizal symbiosis

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Arbuscular mycorrhiza is a symbiosis between plant roots and members of an ancient phylum of soil fungi, the Glomeromycota. While colonizing plant roots, mycorrhizal fungi also form extensive hyphal networks in the soil. The fungus provides water and minerals (mainly nitrogen and phosphorus) to the plant in exchange for photosynthetic sugars. For that the fungus must differentiate highly branched specific structures within root cortical cells called arbuscules (Smith & Read, 2008). Secreted proteins are important regulators of plant - microbe interactions. Microbes secrete proteins that are known to interfere with the plant defense to promote colonization. I am interested in fungal secreted proteins produced by the arbuscular mycorrhizal fungus *Rhizophagus irregularis*, and potentially involved in the regulation of the symbiosis with the host *Medicago truncatula*. We previously identified by RNA-seq *R. irregularis* transcripts encoding secreted proteins that preferentially accumulate in symbiotic tissues (Kamel *et al.*, 2017). Using qRT-PCR, we validated the strong over-expression in mycorrhized roots of *M. truncatula* of a selection of 25 candidates. One protein caught our attention: it is cleaved and produces several copies of a small peptide. This peptide, exogenously applied, stimulates the colonization of *M. truncatula* roots and can be perceived by the fungus itself to induce the transcription of its own gene. To further investigate the role of this peptide, we are currently deciphering whether this peptide may act also at the plant level, for example through an effector-like role. Host-Induced Gene Silencing is currently developed to knock-down the expression of the peptide precursor, and a RNA-seq approach will help understanding which pathways, in either symbiotic partner, are regulated by the peptide.

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Poster14: Gregrory Mouille

Understanding of the oligosaccharide production during *Botrytis cinerea* infection

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The cell wall plays an active role in the defense against necrotrophic pathogens such as Botrytis cinerea. Oligosaccharides accumulate during necrotrophic pathogen-induced cell wall degradation and are major (damage-associated molecular pattern, DAMP) elicitors, which trigger defense responses. Whether or not oligosaccharide signaling molecules are generated depends on the simultaneous presence of host- or pathogen-derived cell wall modifying enzymes. Whether there exist different types of oligosaccharides and how they are perceived remains to be determined. In this context, we first characterised the oligosaccharides released by wild-type *B. cinerea* strain and mutants affected in some pectin-degrading enzymes during infection and presenting different virulences. The eliciting effect of discriminating oligosaccharides and the receptors involved in their perception will be investigated.

Poster15 : Oliver Nagel

The *Xanthomonas* effector protein XopI suppresses the stomatal immunity of tomato

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Our laboratory studies the interaction between *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) and its host plants pepper and tomato. Essential for pathogenicity is the type III secretion system (T3SS) which translocates more than 30 effector proteins (T3Es) into the plant cell cytoplasm. Here, T3Es suppress the plant innate immunity and alter the plant metabolism to the pathogen's advantage. Due to a eukaryotic F-box motif in the N-terminal region, the T3E XopI is supposed to integrate into plant SCF (Skp1-Cullin-F-box protein) complexes which target proteins for ubiquitination. Interaction studies in yeast showed that XopI specifically interacts with one out of 21 *Arabidopsis thaliana* Skp1-like proteins (ASK), suggesting that upon infection, XopI integrates into particular SCF. A yeast-two-hybrid screen with XopI as bait identified five proteins, that presumably are involved in the regulation of stomatal movement. Silencing of two of these potential interactors confirmed that they mediate stomatal closure after PAMP treatment in *Nicotiana benthamiana*. In tomato plants, virulence of *Xcv*85-10Δ*xopI* strains is dramatically reduced. The stomatal aperture is as well reduced, suggesting that XopI is essential for *Xcv* entry into the host plant apoplast. Stomata assays with stable *xopI* transgenic *N. benthamiana* lines showed, that XopI suppresses stomatal closure induced by different treatments, suggesting that XopI maybe affects different pathways of stomatal immunity.

Poster16: Meenu Singla Rastogi

How do plants sense and react to the presence of a bacterial GW effector that targets *Arabidopsis* AGO1 and induces Effector-Triggered-Immunity?

Meenu Singla Rastogi

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Since a wide range of pathogens and pests repeatedly attacks plants, they have evolved a multitude of mechanisms to fight-off and control the invasion by their parasites. In recent years, several plant microRNAs (miRNAs) were found to orchestrate positively or negatively Pattern-Trigger Immunity (PTI). The Arabidopsis miRNA pathway was additionally shown to play a critical role in PTI and, as a corollary, several type-III effectors from *Pseudomonas syringae* pv. *tomato* strain DC3000 (*Pto* DC3000) were found to suppress this small RNA pathway to cause disease. However, the detailed mode of action of such Bacterial Suppressors of RNA silencing (BSRs) remains elusive. Here, we found that the *Pto* DC3000 effector HopT1-1 interacts with, and suppresses the function of, Arabidopsis AGO1, a central component of miRNA-RISC in plants. In addition, we found that plants can recognize the presence of this effector to trigger a potent host-counter-counter defense. I will present the possible mechanisms underlying the sensing of this bacterial effector by host cells. I will also report on the different approaches used to identify and characterize the host immune receptors involved in this process.

Poster17: Simon Saucet

Understanding how the TIR-NB-LRR-PL resistance gene *Ma* activates defense in responses to the root-knot nematodes *Meloidogyne* spp

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The root-knot nematodes (RKNs) *Meloidogyne* spp., represent a global threat for annual and perennial crops causing huge crop losses worldwide. Several genes encoding nucleotide-binding (NB) and leucine-rich repeat (LRR)-containing receptor proteins (NLRs) have been isolated for their ability to provide resistance to RKNs. The NLRs are intracellular immune receptors able to detect secreted pathogenic proteins known as effectors through direct or indirect interaction. Various studies describe the roles of NLRs' protein domains and how they act at intra and intermolecular levels for receptor activation and downstream signalling. However, their way they function is poorly known considering the large number of NLRs identified in plants, the additional domains NLRs often possess, as well as the diversity of NLRs architecture. The gene *Ma*, from the Toll/Interleukin 1 receptor (TIR)-NB-LRR (TNL) family, has been cloned from the plum *Prunus cerasifera* where it provides a high resistance against numerous RKN species. In addition to the core TNL structure, Ma possesses an extension in C-terminal encoded by five repeated exons. Interestingly, a single post-LRR (PL) exon, encoding a domain with conserved motifs, is frequently present in plants TNLs. We are investigating how this repeated PL domain in Ma participates to RKNs resistance in *Prunus*. Using the modular Golden Gate cloning method, we will test *Ma* truncated versions and chimeras for their ability to provide RKNs resistance in transgenic *Prunus* roots. Characterization of Ma's PL domains at intra and intermolecular levels will enable us to reveal the function of this conserved immune region and, ultimately, to contribute to the development of methods to control RKNs infection in crops.

Poster18: Cyril Van Ghelder

TIR-NB-LRR genes in *Prunus* species: insight into the Post-LRR domain

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With the rise of the next generation sequencing and the acquisition of numerous plant genomes and transcriptomes, much effort has been allocated to the localisation and characterisation of resistance (*R*) genes. Therefore, the study of the *R* genes is now a prerequisite to describe a novel sequenced genome. In plants, *R* genes are mostly represented by nucleotide-binding (NB) and leucine-rich repeat (LRR)-containing receptor proteins (NB-LRRs), which recognize secreted pathogen effectors for effector-triggered immunity (ETI). We have studied the sub-family of TIR-NB-LRR (TNL) encoding genes in peach, a species with a small genome, considered as the reference for *Rosaceae*. We have carried out structural and functional annotation to specify the distribution and diversity of this gene family. Our study confirmed that TNL genes were mostly organised in clusters and allowed us to identify genes with unconventional structures. We highlighted the presence of a poorly known C-terminal domain, designated the Post-LRR (PL) domain. By developing specific PL signatures, we showed that this domain is exclusive to TNL genes and is found in most of them (> 66%) in peach as well as in the other tested dicot genomes. Interestingly, in the *Ma* plum gene that confers a complete-spectrum and sustainable resistance to root-knot nematodes (RKNs; *Meloidogyne* spp.), the PL domain displays an atypical and unique five-repeat pattern. Additionally, we recently mapped the *RMja* RKN *R* gene in almond, which displays a TNL architecture similar to *Ma* and is putatively its orthologue. However, *RMja* confers resistance to fewer *Meloidogyne* species. Thus, it represents a great opportunity, using this genetic diversity, to study the molecular determinants involved in the RKN resistance spectrum.

Poster19: Zárate-Chaves

Knowledge of Allele Diversity as a Tool to Improve TALE-Targeted Gene Predictions in the Cassava-Xanthomonas Pathosystem

Zárate-Chaves, C.A.; Osorio, D.; Pérez-Quintero, A.L.; Buitrago-Acosta, M.C.; Jaimes-Niño, L.; Restrepo, S.; López, C., Szurek, B., Bernal, A.

Xanthomonas axonopodis pv. *manihotis* (*Xam*), the causal agent of Cassava Bacterial Blight, is a major threat for cassava farmers. As in many *Xanthomonas* spp., pathogenicity of *Xam* relies on type-III secreted Transcription Activator-Like Effectors (TALEs), a group of proteins that interact with host DNA and induce transcriptional changes of target genes (TGs), including disease-susceptibility genes. Loss-of-susceptibility alleles may be used to create resistant varieties but our knowledge about functional diversity of TALEs in *Xam* is still limited. This work aimed at evaluating the diversity of TALEs among 22 *Xam* strains isolated from different regions and time periods, and predicting potential TGs using *in silico* approaches. We first characterized TALE repertoires by Southern-blot analysis, then, we cloned and sequenced over 50 TALE genes using a strategy based on DNA restriction. Our data show that the *Xam* TALE repertoire is limited in comparison to other Xanthomonads and some effectors are conserved through time. Detailed analyses of TALE sequences showed slight dissimilarities in residues that do not change the affinity for a conserved target. We next grouped TALEs according to their binding abilities and identified candidate TGs using *in silico* predictions. Finally, we performed *in planta* confirmation of induction of these potential TGs using qRT-PCR. In conclusion, the knowledge of TALE diversity is a powerful tool to narrow the search space of TGs and this strategy can facilitate the discovery of TGs, in a faster and cost-effective way.

Poster20: Andrea Paola Zuluaga

Studying the BED protein domain as a new player in plant tolerance to biotic and abiotic stresses

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Identifying new sources of disease resistance and new resistance mechanisms is still a challenge, particularly in Monocots. Moreover, most biological disease resistance pathways identified so far are not efficient under abiotic stress. This is largely due to negative cross-talks between disease resistance and abiotic tolerance. We have demonstrated the role of one protein containing BED domains, called ZBED, in disease resistance in rice against the fungal pathogen *Magnaporthe oryzae*. Quite interestingly, ZBED over-expressor plants also show increased drought tolerance in the field. Thus, this gene represents one of the very few cases where disease resistance and drought tolerance are simultaneously improved. This suggests that ZBED is controlling different biological pathways than those known in disease resistance and drought tolerance. The aim of this work is to understand the molecular function of the ZBED protein and the mechanisms it controls to confer disease resistance. We have demonstrated that ZBED localizes to the nucleus and is able to bind DNA when is transiently expressed in a heterologous system using *Nicotiana benthamiana*. Thus, we hypothesize that ZBED is a nuclear-translocated protein that might act as a transcriptional regulator associated with disease resistance and drought tolerance. In order to test this hypothesis we are currently studying ZBED targets in rice plants, using Chromatin Immunoprecipitation (ChIP) technique.

Additionally, we have determined that ZBED protein interacts with cyclin-dependent kinase inhibitor (KIP1) (Skirpan et al, 2001) and STE11, a homologue of Ste20 kinases (Rawat and Chernoff, 2015). In order to elucidate the regulatory network around ZBED, we have characterized the Kip1 and Ste11 knock-out (KO) mutants in rice and demonstrated that Ste11 KO-mutant is more susceptible to *M. oryzae* than the wild-type. By contrast, Kip1 mutants did not show any phenotype in disease susceptibility or resistance against *M. oryzae*. Next, we want to continue the characterization of these mutants and determine whether they are compromised in drought tolerance.

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