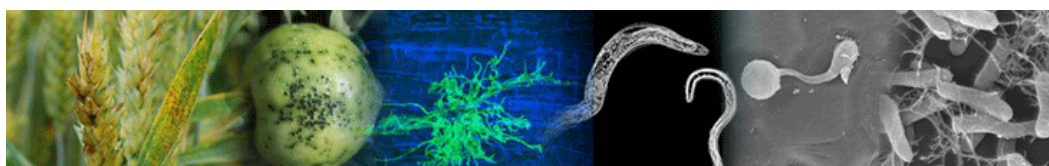




## 3rd Annual Conference of the COST Action Sustain (FA1208)

17<sup>th</sup> – 19<sup>th</sup> February 2016, Banyuls s/ Mer, France

# PROGRAM & ABSTRACT BOOK



LANGUEDOC  
ROUSSILLON  
LA RÉGION MIDI  
PYRÉNÉES



## MEETING OVERVIEW

Wednesday 17th February	Thursday 18th February	Friday 19th February
<b>8:30 - 8:45</b> Welcome		
<b>8:45 - 10:30</b> Session 1 Effectors and Virulence	<b>8:45 - 10:30</b> Session 3 <i>R</i> Genes and Effector-Informed Resistance	<b>8:45 - 10:30</b> Session 4 Effector Targets
<b>Break</b>	<b>Break</b>	<b>Break</b>
<b>11:00 - 12:20</b> Session 1 Effectors and Virulence	<b>11:00 - 12:20</b> Session 3 <i>R</i> Genes and Effector-Informed Resistance	<b>11:00 - 12:20</b> Session 4 Effector Targets  <b>12:20 - 12:30</b> Concluding Remarks
<b>12:30 - 14:00</b> Lunch	<b>12:30 - 14:00</b> Lunch	<b>12:45-14:00</b> Lunch
<b>14:00 -15:25</b> Session 2 Evolution & Diversification	<b>14:00 - 14:30</b> Presentation of Activities of the Sustain Action & Plenary Discussion	
<b>Break</b>	<b>14:30 - 15:30</b> Management Committee Meeting	
<b>16:00 - 17:20</b> Session 2 Evolution & Diversification	<b>14:45 - 16:30</b> Poster Session 2	
<b>17:30 - 19:00</b> Poster Session 1	<b>17:30 - 19:00</b> Visit of the Cave l'Etoile – Banyuls Wine Tasting and Aperitif	
<b>20:00</b> Dinner	<b>20:00</b> Dinner	

**FA1208**

# **Pathogen-informed strategies for sustainable broad-spectrum crop resistance**

**17<sup>th</sup> – 19<sup>th</sup> February 2016, Banyuls s/ Mer, France**

---

## **Organizing Committee**

<b>Audrey Canado</b>	INRA, UMR BGPI, Montpellier, France
<b>Thomas Kroj</b>	INRA, UMR BGPI, Montpellier, France
<b>Nemo Peeters</b>	INRA, UMR LIPM, Toulouse, France
<b>Boris Szurek</b>	IRD, UMR IPME, Montpellier, France

## **Scientific Committee**

<b>Godelieve Gheysen</b>	University of Gent, Belgium
<b>Aska Goverse</b>	Wageningen University, The Netherlands
<b>John Jones</b>	James Hutton Institute, Dundee, UK
<b>Beat Keller</b>	University of Zurich, Switzerland
<b>Thomas Kroj</b>	INRA, UMR BGPI, Montpellier, France
<b>Nemo Peeters</b>	INRA, UMR LIPM, Toulouse, France
<b>Eva Stukenbrock</b>	Max-Planck Institute, Kiel, Germany
<b>Boris Szurek</b>	IRD, UMR IPME, Montpellier, France
<b>Didier Tharreau</b>	CIRAD, UMR BGPI, Montpellier, France
<b>Bart Thomma</b>	Wageningen University, The Netherlands
<b>Hans Thordal Christensen</b>	University of Copenhagen, Denmark
<b>Vivianne Vleeshowers</b>	Wageningen University, The Netherlands

## **Sponsors**



LANGUEDOC  
ROUSSILLON  
**LA RÉGION** MIDI  
PYRÉNÉES



# TABLE OF CONTENTS

CONFERENCE PROGRAM	1
LIST OF POSTERS	5
ABSTRACTS OF ORAL PRESENTATIONS	9
WG1	11
WG3	19
WG4	27
WG2	35
ABSTRACTS OF POSTER PRESENTATIONS	43
PARTICIPANT LIST	94





## WEDNESDAY 17<sup>TH</sup> OF FEBRUARY

**8:30-8:45**      **OPENING & WELCOME BY THE ORGANIZERS**  
**THOMAS KROJ** (CHAIR) & **ASKA GOVERSE** (VICE CHAIR)

### **SESSION 1**      **PATHOGEN EFFECTORS AND VIRULENCE** CHAIRS: GODELIEVE GHEYSEN AND JOHN JONES

**8:45-9:30** **ULLA BONAS** (University of Halle, DE)  
How plant pathogenic bacteria manipulate the plant

**9:30-9:50** **NICO TINTOR** (University of Amsterdam, NL)  
Identification of *Fusarium oxysporum* effectors that are translocated into plant cells during infection

**9:50-10:10** **ALICE MOREL** (INRA, LIPM, Toulouse, FR)  
Tomato targets of the RipHs, conserved type III effectors from *Ralstonia solanacearum*

**10:10-10:30** **SALIM BOURRAS** (University of Zurich, CH)  
Multiple avirulence loci, allele-specific effector recognition, and a pathogen encoded suppressor of avirulence, control the Pm3 race-specific resistance of wheat to powdery mildew

**10:30-11:00**      **BREAK**

**11:00-11:20** **HESHAM GIBRIEL** (University of Wageningen, NL)  
Comparative population genomics combined with genetic mapping promotes effector discovery in the fungal wheat pathogen *Zymoseptoria tritici*

**11:20-11:40** **DIANA NAALDEN** (University of Gent, BE)  
Functional analysis of C-type lectins secreted by the root-knot nematode *Meloidogyne graminicola*

**11:40-12:00** **SUAYIB USTUN** (Leibniz-Institute of Vegetable and Ornamental Crops, DE)  
How plant pathogenic bacteria co-opt the ubiquitin-proteasome system

**12:00-12:20** **MICKAEL QUENTIN** (INRA, Sophia-Antipolis, FR)  
Characterization of root-knot nematode effectors targeting host nuclear functions and identification of their plant targets

**12:30-14:00**      **LUNCH**

### **SESSION 2**      **EFFECTOR EVOLUTION AND DIVERSIFICATION** CHAIRS: EVA STUKENBROCK AND DIDIER THARREAU

**14:00-14:45** **FRANCIS MARTIN** (INRA Nancy, FR)  
Unearthing the Roots of Fungal mycorrhizal Symbioses

**14:45-15:05** **EVA STUKENBROCK** (University of Kiel, DE)  
Recombination rate variation and adaptive evolution in fungal plant pathogens: insight from comparative population genomic studies

**15:05-15:25** **DEBORAH MERDA** (INRA, Angers, FR)  
Evolution of Type 3 effector repertoires in nonpathogenic strains of *Xanthomonas arboricola*

**15:30-16:00**      **BREAK**

**16:00-16:20** **CHARLOTTE VAN DER DOES** (University of Amsterdam, NL)  
Overexpression of either *FTF1* or *SGE1*, encoding conserved transcription factors, induces effector gene expression in *Fusarium oxysporum*

**16:20-16:40** **MAIK RESCHKE** (University of Hanover, DE)  
Insights into the evolution of *Xanthomonas* TAL effectors

**16:40-17:00** **DANIEL CROLL** (ETH Zürich, CH)  
Genome evolution and the genetic basis of virulence in the fungal wheat pathogen *Zymoseptoria tritici*

**17:00-17:20** **JEAN-BENOIT MOREL** (INRA, Montpellier, FR)  
Effector diversity seems to govern local adaptation of the rice blast fungus

**17:30-19:00**      **POSTER SESSION 1**

**20:00**      **DINNER**



## THURSDAY 18<sup>TH</sup> OF FEBRUARY

### **SESSION 3 R GENES & HOST TARGETS FOR RESISTANCE BREEDING AND ENGINEERING**

CHAIRS: VIVIANNE VLEESHOUWERS AND BORIS SZUREK

8:45-9:30 **GREGORY MARTIN** (*Boyce Thomson Institute, USA*)

Using wild relatives of tomato to identify new components of the plant immune system

9:30-9:50 **MATTHIEU JOOSTEN** (*University of Wageningen, NL*)

Cf receptor-like proteins mount plant immunity in a complex with SOBIR1 and SERKs

9:50-10:10 **ABBAS MAQBOOL** (*John Innes Centre, UK*)

Structural basis of effector recognition by a rice NLR immune receptor

10:10-10:30 **XIAO LIN** (*University of Wageningen, NL*)

Identifying and cloning of novel potato immune receptors-recognizing apoplastic effectors of *Phytophthora infestans*—combined effectoromics and receptor enrichment sequencing

10:30-11:00 BREAK

11:00-11:20 **FRANK TAKKEN** (*University of Amsterdam, NL*)

The *Fusarium oxysporum* effector Six8 manipulates plant immunity through association with the transcriptional co-repressors TPL and TPR1

11:20-11:40 **RALF KOEBNIK** (*IRD, Montpellier, FR*)

Targeted promoter editing for rice resistance to *Xanthomonas oryzae* pv. *oryzae* reveals alternative susceptibility pathways among SWEET-inducing TAL effectors

11:40-12:00 **KSENIA KRASILEVA** (*The Sainsbury Laboratory and the Genome Analysis Centre, UK*)

R-gene domain architectures in flowering plants uncover host proteins targeted by the pathogens

12:00-12:20 **ASKA GOVERSE** (*University of Amsterdam, NL*)

The *Rx1-Gpa2* locus in potato: a molecular and genetic framework for engineering novel NLR genes

12:30-14:00 LUNCH

14:00-14:30 **SUSTAIN COST ACTION NETWORK: PRESENTATION AND PLANNED ACTIVITIES**

PLENARY DISCUSSION

14:30-15:30 **SUSTAIN COST ACTION NETWORK: MANAGEMENT COMMITTEE MEETING**

MANAGEMENT COMMITTEE MEMBERS ONLY

14:45-16:30

**POSTER SESSION 2**

17:30-19:00

VISIT OF THE CAVE DE L'ETOILE

BANYULS WINE TASTING AND APERITIF

20:00 DINNER

## FRIDAY 19<sup>TH</sup> OF FEBRUARY

### **SESSION 4 PLANT PROTEINS AND PROCESSES TARGETED BY EFFECTORS**

CHAIRS: ASKA GOVERSE AND NEMO PEETERS

8:45-9:30 **REGINE KAHMANN** (*Max-Planck-Institute Marburg, DE*)

The secreted effector repertoire of smut fungi

9:30-9:50 **SOPHIE MANTELIN** (*The James Hutton Institute, UK*)

NAC transcription factors as susceptibility targets of the potato cyst nematode *Globodera pallida*

9:50-10:10 **LAURENT NOEL** (*LIPM, INRA, Toulouse, FR*)

An adaptor kinase confers expanded recognition specificity to a plant NLR

10:10 -10:30 **RENIER VAN DER HOORN** (*University of Oxford, UK*)

Manipulation of apoplastic hydrolases by *Pseudomonas syringae*

10:30-11:00 BREAK

11:00-11:20 **MAELLE JAOUANNET** (*The James Hutton Institute, UK*)

Plant Histone H3 Methyltransferases targeted by an aphid effector: a regulatory strategy for the suppression of Arabidopsis defences?

11:20-11:40 **YASIN DAGDAS** (*The Sainsbury Laboratory, UK*)

Subversion of autophagy by the Irish famine pathogen *Phytophthora infestans*

11:40-12:00 **NURIA SANCHEZ-COLL** (*CIRAD, BARCELONA, SP*)

The effector AWR5 from the plant pathogen *Ralstonia solanacearum* is an inhibitor of the TOR signaling pathway

12:00-12:20 **JAN SCHIRAWSKI** (*University of Aachen, DE*)

Identification and functional analysis of a fungal effector suppressing apical dominance in maize

12:20-12:30 **CONCLUDING REMARKS AND CLOSURE OF THE CONFERENCE**

12:30-14:00 LUNCH



## POSTERS

Poster N°	First Name	Name	Abstracts
1	Philip	ALBERS	HopZ1a targets a remorin protein implicated in membrane-associated defence signalling
2	Carolina	AGUILERA GALVEZ	Specificity of recognition to <i>P. infestans</i> AVR2 effector family mediated by <i>Solanum</i> R gene families
3	Nuno Felipe	ALMEIDA	The pursuit of effector targets in <i>Lathyrus cicera</i> rust resistance QTLs
4	Lander	BAUTERS	Chorismate mutase and isochorismatase, two parasitism proteins of the nematode <i>Hirschmanniella oryzae</i> , increase susceptibility of rice to nematode infection
5	Janos	BINDICS	Identification of <i>Ustilago maydis</i> Effectors Targeting Hormonal Signaling
6	Eran	BOSIS	Identification of New <i>Xanthomonas</i> Type III Effectors by a Bioinformatic Approach
7	Klaas	BOUWMEESTER	Lectin receptor kinases in microbial recognition and plant immunity
8	María Angeles	CASTILLEJO	Understanding pea resistance mechanisms in response to <i>Fusarium oxysporum</i> through proteomic analysis
9	James	COCKRAM	EffectaWheat : An Effector- and Genomics-Assisted Pipeline for <i>Necrotrophic Pathogen</i> Resistance Breeding in Wheat
10	Anna	COLL	StERF, an Ethylene Response Factor involved in potato defence response to PVY
11	Karine	DE GUILLEN	<i>Magnaporthe oryzae</i> effectors AVR-Pia and AVR1-CO39 Reveal Structural Homology
12	Amalia	DIAZ GRANADOS	Exploring the role of Rbp-1 in <i>Globodera pallida</i> parasitism
13	Rowena	DOWNIE	Mapping the wheat <i>Snn1</i> locus conferring sensitivity to the <i>Parastagonospora nodorum</i> necrotrophic effector SnTox1 using an eight founder multi-parent advanced generation intercross population
14	Abdelnaser	ELASHRY	Investigation of <i>Heterodera schachtii</i> transcriptome to identify putative effectors
15	Maria Raffaella	ERCOLANO	Comparative genomic approaches for investigating <i>Solanaceae</i> defence system
16	Lennart	ESCHEN LIPPOLD	Bacterial effector-mediated suppression of PAMP-induced defence signalling
17	Sebastian	EVES VAN DEN AKKER	The evolution and diversification of novel biosynthetic function's in effectors: a basis for specific pathogen-informed drug design?
18	Bruno	FAVERY	Comprehensive Transcriptome Profiling of Root-knot Nematodes During Plant Infection and Characterization of Species-Specific Traits
19	Sara	FONDEVILLA	In planta identification of putative pathogenicity factors from the chickpea pathogen <i>Ascochyta rabiei</i>

20	Sharon	GARRIDO	Functional analyses of putative determinants of host specialization and pathogenicity in <i>Zymoseptoria tritici</i>
21	Valerie	GEFFROY	Molecular basis and origin of <i>Co-x</i> , an atypical disease resistance gene to anthracnose in common Bean
22	Laurence	GODIARD	Exploitation of the knowledge on oomycete effectors to drive the discovery of durable disease resistance in cultivated plants: the case of <i>Plasmopara halstedii</i> , the agent of sunflower downy mildew
23	Rafal	HOSER	Analysis of evolutionary adaptation of HopQ1 effector from <i>Pseudomonas syringae</i> to given plant host species
24	Marijn	KNIP	Studying the link between DNA-damage and NLR-mediated immune responses
25	Paolo	IOVIENO	Identification of candidate MLO powdery mildew susceptibility genes in <i>Cucurbita Pepo</i> and functional characterization in tomato
26	Jakub	KWIATKOWSKI	Elucidation of mechanisms underlying virulence function of <i>Pseudomonas syringae</i> HopQ1 effector in plant cells
27	Marc-Henri	LEBRUN	Wheat Effector Assisted Breeding for Resistance to Fungal Pathogens (WEAB)
28	Jana	LIBANTOVA	Chitinase of <i>Drosera rotundifolia</i> in transgenic tobacco protein extracts suppressed the growth of <i>Fusarium poae</i> in hyphal extension assay
29	Fabien	LONJON	Comparative Secretome Analysis of <i>Ralstonia solanacearum</i> Type 3 Secretion-Associated Mutants Reveals a Fine Control of Effector Delivery, Essential for Bacterial Pathogenicity
30	Diego	LOPÉZ MARQUEZ	MicroRNA-mediated regulation of R genes involved in the plant response against <i>Pseudomonas syringae</i>
31	Takaki	MAEKAWA	Convergent targeting of a host-signalling pathway by unrelated pathogen effectors and their surveillance by allelic immune receptors
32	Johana C	MISAS VILLAMIL	A fungal effector reveals new mechanisms in the inhibition of <i>Cysteine proteases</i>
33	Hélène	MISSONNIER	Study of the genomic diversity of <i>Verticillium dahliae</i> found in naturally infested sunflower fields
34	Diana	ORTIZ	The molecular bases of recognition of the <i>M. oryzae</i> effector protein AVR-Pia by the rice immune receptor RGA5
35	Javier	PALMA GUERRERO	Using comparative transcriptomics to identify new virulence factors in the wheat pathogen <i>Zymoseptoria tritici</i>
36	Nemo	PEETERS	Functional Assignment to Positively Selected Sites in the Core Type III Effector RipG7 from <i>Ralstonia solanacearum</i>
37	Hélène	PIDON	Insight into the diversity of plant resistance mechanisms to viruses through the Rice-Rice yellow mottle virus pathosystem
38	Marc	PLANAS	Determining tomato apoplast responses to <i>Ralstonia solanacearum</i> by Activity-based protein profiling

39	Loris	PRATX	Identification of epigenetic marks in the plant parasitic root-knot nematode <i>Meloidogyne incognita</i>
40	Dov	PRUSKY	Carbon regulation of environmental pH by secreted small effecting molecules that modulate pathogenicity in phytopathogenic fungi
41	Liliya	PYLYPENKO	Development and validation of the marker for the NPR1-like <i>Fusarium</i> head blight resistance gene
42	Dina	RAATS	Rapid isolation of new stripe rust resistance variants in cultivated wheat
43	Amey	REDKAR	Elucidating the mechanistic basis of Albugo candida mediated Immune-suppression by CCG effectors
44	Javier	RUIZ ALBERT	Effector-mediated mechanisms of plant defence evasion in <i>Pseudomonas syringae</i>
45	Andrea	SANCHEZ VALLET	Exploring genetic diversity to identify virulence factors in the wheat pathogen <i>Zymoseptoria tritici</i>
46	Guido	SESSA	The <i>Xanthomonas</i> effector XopAU is an active protein kinase that manipulates host MAP kinase signaling to promote disease
47	Alan	SCHULMAN	Fine-mapping of the <i>Rpt5</i> net blotch resistance gene region in barley
48	Waldemar	SKOWRON	Tomato <i>R</i> -genes are targeted by miRNA during nematode pathogenesis
49	Yin	SONG	Characterization of <i>Verticillium wilt</i> resistance genes from <i>Nicotiana glutinosa</i> and <i>Humulus lupulus</i> reveals ancient origin of Ve1 immune receptor homologs in plants
50	Jana	STREUBEL	Dissection of TALE-mediated transcriptional enhancement
51	Octavina	SUKARTA	Identification and functional analysis of novel regulatory components of the potato NLR immune receptors Rx1 and Gpa2
52	Magdalena	SWIECICKA	Novel tomato miRNAs are the predominant component of RNAi upon <i>Globodera rostochiensis</i> infection
53	Boris	SZUREK	Functional analysis of the TALome of african <i>Xanthomonas oryzae</i> pv. <i>oryzae</i> reveals a new bacterial leaf blight susceptibility gene candidate
54	Suayib	USTUN	How plant pathogenic bacteria co-opt the ubiquitin-proteasome system
55	Fabienne	VAILLEAU	HpaP modulates type 3 effector secretion in <i>Ralstonia solanacearum</i> and plays an essential role in virulence
56	Aranka	VAN DER BURGH	The receptor-like kinase SOBIR1_'EVR is essential for immune signalling downstream of Cf-4
57	Michel	VAN THOURNOUT	Two <i>R</i> -genes at a single genetic locus confer resistance to Clubroot in Oilseed Rape



# **ABSTRACTS OF ORAL PRESENTATIONS**





# Wednesday 17<sup>th</sup> of February 2016

## Session 1

### **PATHOGEN EFFECTORS AND VIRULENCE**

Ulla BONAS

#### **How plant pathogenic bacteria manipulate the plant**

*Department of Genetics, Martin Luther University Halle-Wittenberg, Halle, Germany*

Pathogenicity of most Gram-negative plant-pathogenic bacteria depends on the type III secretion (T3S) system which translocates effector proteins (T3Es) into the plant cell cytosol. We study the interaction between *Xanthomonas campestris* pv. *vesicatoria* (Xcv) and its host plants pepper and tomato. In susceptible plants, T3Es interfere with host cell processes to the benefit of the pathogen and allow its proliferation in the apoplastic space of the plant leaf mesophyll. In resistant plants, single resistance genes mediate recognition of individual T3Es thus often inducing a hypersensitive response (HR), a rapid and localized programmed cell death. Xcv injects more than 25 different T3Es into the plant cell, termed Avr (avirulence protein) or Xop (Xanthomonas outer protein). Among the T3Es from Xcv are plant immunity suppressors, cell death inducers, a ubiquitin ligase, a transcription factor and proteins of unknown function. Selected T3Es will be discussed.

## **Identification of *Fusarium oxysporum* effectors that are translocated into plant cells during infection**

Nico Tintor<sup>1</sup>, Peter van Dam<sup>1</sup>, Libera Lo Presti<sup>2</sup>, Regine Kahmann<sup>2</sup> and Martijn Rep<sup>1</sup>

*1 Molecular Plant Pathology, University of Amsterdam, the Netherlands*

*2 Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*

Fungi are widespread colonizers of plants, and some can also cause devastating plant diseases. Colonization success largely depends on the ability to manipulate the host plant, often achieved via effectors, secreted proteins that act either inside plant cells or in the apoplast. *Fusarium oxysporum* is a soil inhabiting fungus that can infect many plant species via the roots. Its effectors were previously characterized as small, secreted proteins that accumulate in the xylem vessels during infection. For several *F. oxysporum* effectors a contribution to disease development has been demonstrated, but the underlying virulence mechanisms remain unknown. Using the *Arabidopsis* – *F. oxysporum* pathosystem we are aiming to identify the functional sites and virulence mechanisms of these effectors. We generated a shortlist of ca. 20 candidate effectors from an *Arabidopsis* infecting *F. oxysporum* strain, based on homology to known effectors and presence of a ‘miniature impala’ (mimp) transposable element in the promoter. To investigate which of these effectors are translocated into living cells during infection, we apply an in planta biotinylation assay. Candidate effectors were C-terminally fused to a short tag that serves as a biotin-acceptor site inside plant cells and transformed into *F. oxysporum*. The tested effectors show either strong or very weak/undetectable biotinylation, indicating predominant intracellular vs. apoplastic localization. Furthermore, a set of effectors was affinity purified from infected roots, and currently mass spectrometry is applied to identify interacting proteins. Putative interactors may include the virulence target(s), but also proteins mediating effector trafficking or uptake.

## **Tomato targets of the RipHs, conserved type III effectors from *Ralstonia solanacearum***

Alice Morel<sup>1</sup>, Patrick Barberis<sup>1,2</sup>, Xavier Barlet<sup>1,2</sup>, Gaofei Jiang<sup>1,2</sup>, Fabien Lonjon<sup>1,2</sup>, Fabienne Vailleau<sup>1,2,3</sup>, Stéphane Genin<sup>1,2</sup> and Nemo Peeters<sup>1,2</sup>

*1 INRA, Laboratoire des Interactions Plantes Micro-organismes (LIPM), UMR441, Castanet-Tolosan, France*

*2 CNRS, Laboratoire des Interactions Plantes Micro-organismes (LIPM), UMR2594, Castanet-Tolosan, France*

*3 Université de Toulouse; INP; ENSAT; 18 chemin de Borde Rouge, 31326 Castanet-Tolosan, France*

*Ralstonia solanacearum* is a soil-born bacterium causing the bacterial wilt disease on a large number of plant hosts. This disease is spread worldwide and affects different crops, including tomato, mostly in warm climate countries. One of the major virulence determinants is the type III secretion system that enables the bacterium to directly inject proteins (the Type III effectors or T3Es) into the host cells. We demonstrated that the RipH paralogous family (RipH1, 2, 3) are required for the virulence of *R. solanacearum* on several host plants with a genetic functional redundancy on tomato (the mutants possessing one of these 3 effectors have the same pathogenicity than the wild bacterium). Using yeast-two-hybrid screening we have identified several tomato targets of these T3Es. Interestingly, some of these targets are common to RipH1 and RipH3, including several transcription factors. Linked with the nuclear localization of the RipH1, 2, 3, we are investigating the role of these effectors in the manipulation of the host transcriptional regulation involved in defenses pathways against the bacterium. I will present our current advance on the analysis of the effect of the RipH on these tomato targets, in a global effort to understand the contribution of these T3Es to the virulence mechanisms of the bacterium

## **Multiple avirulence loci, allele-specific effector recognition, and a pathogen encoded suppressor of avirulence, control the Pm3 race-specific resistance of wheat to powdery mildew**

Salim Bourras<sup>1</sup>, Kaitlin Elyse McNally<sup>1</sup>, Roi Ben-David<sup>1,2</sup>, Francis Parlange<sup>1</sup>, Stefan Roffler<sup>1</sup>, Coraline Rosalie Praz<sup>1</sup>, Simone Oberhaensli<sup>1</sup>, Fabrizio Menardo<sup>1</sup>, Daniel Stirnweis<sup>1,3</sup>, Zeev Frenkel<sup>4</sup>, Luisa Katharina Schaefer<sup>1</sup>, Simon Flückiger<sup>1</sup>, Georges Treier<sup>1</sup>, Gerhard Herren<sup>1</sup>, Abraham B. Korol<sup>4</sup>, Thomas Wicker<sup>1</sup> and Beat Keller<sup>1</sup>

*1 Institute of Plant Biology, University of Zurich, Zollikerstrasse 107, CH-8008 Zürich, Switzerland*

*2 Current address: Institute of Plant Sciences, Agricultural Research Organization, Volcani Center, 50250 Bet Dagan, Israel*

*3 Current address: KWS Saat AG, Grimsehlstrasse 31, 37555 Einbeck, Germany*

*4 Institute of Evolution, University of Haifa, Mount Carmel, 31905 Haifa, Israel*

In cereals, several mildew resistance genes occur as large allelic series. In wheat, 17 functional Pm3 alleles confer agronomically important race-specific resistance. While the molecular basis of race-specificity is well characterized in wheat, little is known about allele-specific avirulence in powdery mildew since no avirulence gene for Pm3 was cloned. Here, we dissected the genetic network of virulence/avirulence towards six Pm3 alleles and found that it is controlled by three major Avr loci, with a common locus<sub>1</sub>, encoding for a candidate effector gene, and involved in all AvrPm3-Pm3 interactions. We cloned the effector gene AvrPm3a2/f2 from locus<sub>2</sub> which is specifically recognized by the Pm3a and Pm3f alleles. Specificity was demonstrated by induction of an allele-specific hypersensitive response in transient assays in *Nicotiana benthamiana* and in wheat. Gene expression analysis of locus<sub>1</sub> encoded effector gene Bcg1 and AvrPm3 a2/f2 revealed significant quantitative differences between isolates, indicating that in addition to protein polymorphisms, expression levels play a role in virulence/avirulence. We propose the “Avr-R-Svr” model involving three components for race-specificity: an allele-specific avirulence effector (Avr), a resistance gene allele (R), and a pathogen-encoded suppressor of avirulence (Svr). Thus, whereas specificity is controlled by a genetically simple allelic series in wheat, recognition and suppression of recognition on the pathogen side is more complex, allowing flexible evolutionary responses and adaptation to resistance genes.

## **Comparative population genomics combined with genetic mapping promotes effector discovery in the fungal wheat pathogen *Zymoseptoria tritici***

Hesham Gibriel<sup>1</sup>, Amir Mirzadi Gohari<sup>2</sup>, Lamia Auoini<sup>2</sup>, Harold J.G. Meijer<sup>2</sup>, Gert H.J. Kema<sup>2</sup>, Bart P.H.J. Thomma<sup>1</sup> and Michael F. Seidl<sup>1</sup>

*1 Wageningen University, Wageningen, the Netherlands*

*2 Wageningen University and Research Center, Plant Research International, 6700AB Wageningen, The Netherlands*

*Zymoseptoria tritici*, the causal agent of *septoria tritici* blotch, poses major threats for wheat production worldwide. Different *Z. tritici* isolates exhibit a high level of wheat-cultivar specificity, which is mediated by host recognition that renders specific *Z. tritici* isolates avirulent. Even though a high-quality genome assembly for the *Z. tritici* reference isolate IPO323 is available, only few secreted proteins that promote fungal virulence –so called effectors- have thus far been identified. In addition, genes that contribute to wheat-cultivar specificity, for example by host recognition of their products, remain unknown. We suggest that this could be at least partially explained by the incompleteness of the currently annotated reference genome. Therefore, we generated a novel, high-quality gene annotation for the *Z. tritici* IPO323 genome, yielding ~3,000 newly predicted genes. By exploiting this resource together with publicly available transcriptome data, we determined multiple novel effector candidates that display induced expression during wheat infection. Subsequently, we combined genetic mapping with high-resolution genotyping-by-sequencing of multiple avirulent or virulent *Z. tritici* isolates, and identified candidate genes that may contribute to wheat-cultivar specificity. Functional analysis will be performed to confirm the contribution of candidate genes to wheat-cultivar specificity. Here, we describe an effective approach to determine effector genes, and in particular genes that contribute to wheat-cultivar specificity, which will provide novel insights into the mechanism of host recognition and the establishment of resistance in wheat.

## **Functional analysis of C-type lectins secreted by the root-knot nematode *Meloidogyne graminicola***

Diana Naalden<sup>1</sup>, Annelies Haegeman<sup>2</sup>, Silke Nowak<sup>1</sup>, Nguyen Xuan Huy<sup>1</sup>, Romnick Latina<sup>1</sup>, Godelieve Gheysen<sup>1</sup>

<sup>1</sup> Department of Molecular Biotechnology, Ghent University, Coupure links 653, 9000 Ghent, Belgium

<sup>2</sup> Plant Sciences Unit, Institute for Agricultural and Fisheries Research (ILVO), Caritasstraat 21, B-9090 Melle, Belgium

Rice (*Oryza sativa* L.) is a staple food mainly produced in Asia. Scarcity of fresh water causes a shift to aerobic rice cultivation which benefits the distribution and infection of *Meloidogyne graminicola*, the most damaging root-knot nematode of rice. *M. graminicola* has a sedentary life style and therefore developed complex strategies to invade and survive in the roots for a long period. Effectors secreted in the plant by the nematode are essential for successful parasitism. In a previous study, potential effector genes expressed in the pre-parasitic stages were identified using 454 sequencing technology and in situ hybridization (Haegeman et al., 2012). This study indicated that, like other plant parasitic nematodes, *M. graminicola* secretes C-type lectins. However, little is known about the function of these proteins in plant parasitism. In our research, we focus on two C-type lectins that are expressed in the subventral glands of the nematode. Assays triggering the early defense response of the plant suggest that these lectins have a role in the suppression of PTI signaling. Further research is now being done to gain more insight in the mechanism behind this suppression.

## **How plant pathogenic bacteria co-opt the ubiquitin-proteasome system**

Suayib Üstün<sup>1</sup>, Arsheed Sheikh<sup>3</sup>, Alex Jones<sup>3</sup>, Wolfgang Hoehenwarter<sup>4</sup>, Vardis Ntoukakis<sup>3</sup> and Frederik Börnke<sup>1,2</sup>

*1 Leibniz-Institute for Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany*

*2 Institute for Biochemistry and Biology, University of Potsdam, Germany*

*3 School of Life Sciences University of Warwick, Coventry, United Kingdom*

*4 Leibniz Institute for Plant Biochemistry, Halle (Saale), Germany*

Plant pathogenic bacteria translocate about 30 type-III effector proteins (T3E) into the host cell to cause disease. These T3Es manipulate processes including secretion, the ubiquitin-proteasome system (UPS) and gene expression. Evidence is emerging that manipulation of the UPS might be an effective and widespread virulence strategy of bacterial invaders to promote pathogenesis. In line with this, we could show that *Xanthomonas* T3E XopJ promotes virulence through the inhibition of the proteasome and a resultant suppression of SA-dependent defense. XopJ acts as a cysteine protease to degrade proteasomal subunit RPT6 triggering proteasome malfunction. Consequently, XopJ-mediated suppression of the proteasome impairs the proteasomal turnover of NPR1 leading to its accumulation. Preliminary analysis of the XopJ-induced ubiquitylome revealed candidates implicated in UPS, vesicle trafficking and calcium signalling. In addition, we show that *Pseudomonas syringae* also inhibits proteasome activity in a type-III secretion dependent manner. A systematic screen for T3Es from *Pseudomonas* for their ability to interfere with proteasome activity revealed HopM1, HopAO1 and HopG1 as candidates. Identification of proteins interacting with HopM1 by mass-spectrometry indicate that HopM1 resides in a complex together with several E3 ubiquitin ligases and proteasome subunits, supporting the observation and hypothesis that HopM1 is ubiquitylated in plants to associate with the proteasome leading to its inhibition. Further functional characterization of other *Xanthomonas* T3Es unveiled effectors localized in the nucleus that interact with UPS components to stabilize transcription factors. Thus, the manipulation of the host cell proteasome is an efficient virulence mechanism of phytopathogens that evolved different effector repertoires.



## **Characterization of root-knot nematode effectors targeting host nuclear functions, and identification of their plant targets**

Mickael Quentin, Truong, N.M, Nguyen, C.-N., Mejias, J., Magliano, M., Perfus-Barbeoch, L., DaRocha, M., Danchin, E.G., Abad, P. Favory, B

*INRA, Univ. Nice Sophia Antipolis, CNRS, UMR 1355-7254 Institut Sophia Agrobiotech, 06900 Sophia Antipolis, France*

Plant parasitic nematodes are microscopic worms, the most damaging species of which have adopted a sedentary lifestyle within their hosts. These obligate endoparasites are biotrophs that induce the differentiation of root cells into hypertrophied, multinucleate feeding cells. Effectors synthesized in the esophageal glands of the nematode are injected into the plant cells via the syringe-like stylet and may be required to modulate many aspects of plant cell morphogenesis and physiology leading to the establishment of the feeding giant cells. In a search for *Meloidogyne incognita* effectors targeting to the giant cell nuclei, we used bioinformatics and comparative genomics on EST and NGS datasets to identify genes encoding proteins potentially secreted upon the early steps of infection. We identified genes specifically expressed in the esophageal glands of parasitic juveniles that encode predicted secreted proteins and have a Nuclear Localization Signal and/or a DNA-Binding Domain. In planta nuclear localization of these putative effectors was confirmed using tobacco agro-infiltration, and siRNA soaking was used to silence these genes and study their role during parasitism. Using a yeast-two-hybrid approach and BiFC, we aim at identifying host nuclear functions manipulated by these effectors.

## Session 2

# EFFECTOR EVOLUTION AND DIVERSIFICATION

Francis MARTIN

### **Unearthing the Roots of *Fungal mycorrhizal* Symbioses**

Francis M. Martin<sup>1</sup>, Annegret Kohler<sup>1</sup>, Alan Kuo<sup>2</sup>, László G Nagy<sup>3</sup>, Emmanuelle Morin<sup>1</sup>, Igor V Grigoriev<sup>2</sup>, David Hibbett<sup>3</sup>  
& Mycorrhizal Genomics Initiative (MGI) Consortiumx

<sup>1</sup> INRA, Laboratory of Excellence ARBRE, UMR 1136, Champenoux, France

<sup>2</sup> US Department of Energy Joint Genome Institute, Walnut Creek, California, USA

<sup>3</sup> Department of Biology, Clark University, Worcester, Massachusetts, USA

Forest health, productivity and sustainability depend on above- and below-ground microbial associations to exchange nutrients, recycle carbon, and sustain diseases and harsh environmental conditions. Fungi are often described as either saprotrophs, which degrade complex organic substrates, or biotrophs, which obtain carbon compounds from living hosts. Among the latter, ectomycorrhizal (ECM) fungi provide crucial ecological services in interacting with most forest trees. They are portrayed as mutualists trading plant host photoassimilates for nutrients and having limited capacity to decompose soil lignocellulose. An improved understanding of the role of ECM fungi and their evolutionary adaptive history in the face of changing environmental conditions will create tools to predict how they are likely to adapt to future climate change. A major goal of mycorrhizal studies is also to define the symbiosis in molecular terms, i.e. to identify the 'symbiosis genes' that encode the molecules that mediate and regulate symbiosis development and the coordinated symbiotic metabolic pathways. To identify the genetic innovations that led to convergent evolution of the mycorrhizal lifestyle from ancestral saprotrophic species, we have conducted the first broad, comparative phylogenomic analysis of mycorrhizal fungi, drawing on 50 genomes from ECM, orchid (ORM), ericoid (ERM) and related saprotrophic fungi. The analyses of these genomes suggested that mycorrhizal symbioses evolved from ecologically diverse decayer precursors and radiated in parallel, following the origins of their host-plant lineages. Polyphyletic evolution of the ECM lifestyle is marked not only by convergent losses of different components of the ancestral saprotrophic apparatus, such as plant cell wall degrading enzymes, but also by rapid genetic turnover in symbiosis-induced orphan genes, some of which may reflect lineage-specific functional innovations, such as effector-like mycorrhiza-induced small secreted proteins (MiSSPs). In contrast, ERM and ORM fungi retained an extensive decay apparatus that is probably exploited indirectly by the plant for carbohydrate supply, thus explaining their known saprotrophic ability. Understanding the driving forces and the molecular mechanisms behind these gene gains and losses remain a big challenge for future research. By combining genome sequences with rigorous metabolic studies, and landscape-scale metatranscriptomics of soil dynamics *in situ*, we are entering a time where linking the presence, composition and abundance of soil mycorrhizal communities with important soil processes and forest productivity at an ecosystem scale is possible.

## **Recombination rate variation and adaptive evolution in fungal plant pathogens: Insight from comparative population genomic studies**

Julien Dutheil, Eva Stukenbrock

*Max Planck for Evolutionary Biology, August-Thienemann-Str. 2, 24306 Plön, Germany*

Antagonistic co-evolution between pathogens and their hosts can drive rapid adaptive changes in both partners. We aim to understand the underlying mechanisms driving rapid adaptation in two closely related fungal plant pathogens *Zymoseptoria tritici* (wheat pathogen) and *Z. ardabiliae* (wild grass pathogen). We previously showed a significantly higher effective population size in *Z. tritici* in spite of strong directional selection pressure imposed to the pathogen in the wheat field. Also we showed a strong impact of natural selection on genome evolution in *Z. tritici*. A high efficacy of selection may be mediated by recombination. We applied a population genomics approach to infer genome-wide recombination maps in *Z. tritici* and *Z. ardabiliae*. Comparing mean recombination rates of coding and non-coding sequences, we find significantly higher recombination rates in coding sequences implying a central role of recombination in gene evolution. Some genes are located in recombination hotspots further supporting a central role of recombination in gene evolution. In both species recombination rate varies across chromosomes. We correlated recombination maps in the syntenic genomes of *Z. tritici* and *Z. ardabiliae*. Some regions have conserved patterns of recombination while others show highly different patterns. We conclude that patterns of recombination rate evolve in *Zymoseptoria* allowing orthologous genes to evolve at highly different rates in *Z. tritici* and *Z. ardabiliae*...

## **Evolution of Type 3 effector repertoire in nonpathogenic strains of *Xanthomonas arboricola***

Déborah Merda, Martial Briand, Marie-Agnès Jacques, Marion Fischer-Le Saux

INRA, UMR 1345 IRHS, Beaucauzé, France

Acquisition of virulence factors through horizontal gene transfers (HGT) can modify the pathogenic profile of strains and lead to the emergence of new diseases. Within bacteria, HGTs are more frequent for individuals which are phylogenetically close. As pathogenic and nonpathogenic individuals can coexist within the same species, it is very likely that they exchange genetic material when in sympatry. Nonpathogenic strains are defined as strains, which are unable to cause disease on their host of isolation. In order to understand pathogen emergence in agroecosystem, it is important to identify the evolutionary mechanisms, which led to the installation of these two types of populations. The species *Xanthomonas arboricola* encompasses nine pathovars (an infrasubspecific division grouping strains causing the same disease on the same host range) as well as nonpathogenic strains. The repertoire analyses of type 3 effectors (T3Es) showed that the emergences of the three successful pathovars (*X. arboricola* pv. *juglandis*, *X. arboricola* pv. *pruni* and *X. arboricola* pv. *corylina*) was linked to the acquisition of nine T3Es whereas nonpathogenic strains and unsuccessful pathovars keep the ancestral repertoire or lost these factors during the evolutionary history of *X. arboricola*. Comparative genomics on 45 genome sequences representing successful pathovars and non-pathogenic and unsuccessful pathovars of *X. arboricola* was performed. On the one hand, we analyzed the evolution of type 3 secretion system, and its presence / absence in nonpathogenic strains. On the other hand, we analyzed the genomic environments of T3Es to determinate the molecular mechanisms responsible for gene deletion or acquisition.

**Overexpression of either FTF1 or SGE1, encoding conserved transcription factors, induces effector gene expression in *Fusarium oxysporum***

Charlotte Van Der Does<sup>1</sup>, Ally Yang<sup>2</sup>, Like Fokkens<sup>1</sup>, Sarah M. Schmidt<sup>1</sup>, Ernst-Jan Eggers<sup>1</sup>, Joanna M. Lukasiewicz<sup>1</sup>, Léon Langereis<sup>1</sup>, Tim Hughes<sup>2</sup> and Martijn Rep<sup>1</sup>

*1 University of Amsterdam, the Netherlands*

*2 University of Toronto, Canada*

In the tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici*, most known effector genes reside on an accessory chromosome that can be exchanged between strains through horizontal transfer. Expression of these effector genes is massively upregulated upon infection and requires SGE1, a transcription factor encoded on the core genome [1]. The accessory chromosome itself also contains 13 predicted transcription factor genes. To see if the accessory chromosome could be transcriptionally semi-autonomous, we investigated the possible function of the 'accessory' transcription factor genes in effector gene expression. Of all transcription factor genes on the accessory chromosome except one, there is a homolog on the core genome. We obtained DNA binding data for nine of these transcription factors using oligonucleotide arrays. The binding sites for homologous transcription factors were in all cases highly similar, indicating no diversification in recognition specificity has occurred. However, a majority of these DBSs is enriched on the accessory chromosomes. Overexpression of all accessory transcription factor genes revealed that only FTF1, its core homolog FTF2, and SGE1 are able to induce expression of the SIX1 effector gene. Also, the putative DBS of these transcription factors is enriched among genes upregulated during infection. RNAseq analysis of the overexpression strains revealed that FTF1, FTF2 and SGE1 strongly induce a similar set of plant-responsive genes on the accessory chromosome including almost all effector genes.

## **Insights into the evolution of *Xanthomonas* TAL-Effectors**

Maik Reschke<sup>1</sup>, Jan Grau<sup>2</sup>, Annett Erkes<sup>2</sup>, Jana Streubel<sup>1</sup>, Richard D. Morgan<sup>3</sup>, Geoffrey G. Wilson<sup>3</sup>, Ralf Koebnik<sup>4</sup>, Jens Boch<sup>1</sup>

*1 Institute of Plant Genetics, Leibniz University Hannover, Herrenhäuser Straße 2, 30419 Hannover, Germany*

*2 Institute of Computer Science, Martin Luther University Halle–Wittenberg, Von-Seckendorff-Platz 1, 06120 Halle, Germany*

*3 New England Biolabs Inc., Ipswich, MA 01938-2723, USA*

*4 UMR 186 IRD-UM2-Cirad "Résistance des Plantes aux Bioagresseurs", BP 64501, 34394 Montpellier cedex 5, France*

Plant-pathogenic *Xanthomonas* bacteria use transcription activator-like effectors (TALEs) as important virulence factors that bind to the promoter of plant genes and activate their transcription. *Xanthomonas* infection results in a substantial yield loss for many crop plants including rice. The binding domain of TALEs consists of tandem repeats containing two hyper-variable amino acids, which are called repeat variable di-residue (RVD). Each RVD recognizes one nucleotide of its target DNA and the consecutive array of RVDs determines TALE target specificity. Rice-pathogenic *Xanthomonas oryzae* contain many TALE genes, but the full repertoire of these effectors in different strains is largely unknown. We address this by de novo genome sequencing of *X. oryzae* strains using PacBio. This method is uniquely able to solve the highly repetitive sequence of TALEs. In consequence, we developed AnnoTALE, an application for annotating TALEs in *Xanthomonas* genomes, for analyzing their putative target genes, and for clustering TALEs into classes based on the similarity of their RVD sequences. Building classes of TALEs from published and four newly sequenced *Xanthomonas* genomes helped us to gain new insights into the evolutionary and functional biology of TALEs. Comparing TALE repertoires we identified some highly conserved TALE genes as well as unique ones or TALEs that originate from recombination between different TALEs. TALEs are typically clustered and we defined nine TALE loci of which some seem to evolve by genomic rearrangements. To understand the contribution of individual *X. oryzae* TALEs to virulence, we predict their targets and perform RNA-Seq to identify induced rice genes.

## **Genome evolution and the genetic basis of virulence in the fungal wheat pathogen *Zymoseptoria tritici***

Fanny Hartmann, Andrea Sánchez Vallet, Daniel Croll

*Institute of Integrative Biology, ETH Zurich, Switzerland*

The fungal pathogen *Zymoseptoria tritici* causes a significant disease on wheat called Septoria tritici blotch (STB). Pathogen populations show extraordinary evolutionary potential to adapt to changes in the environment, host resistance genotypes and fungicides. The main drivers of rapid evolution are thought to be frequent sexual reproduction and high dispersal capabilities. But despite the ubiquity of evidence for rapid turnover occurring in fungal populations, little is known how the structure of the genome influences the evolution of genetic variation. We first aimed to identify polymorphism linked to variation in virulence on two different wheat cultivars. For this, we performed whole-genome resequencing of 130 isolates and identified 751'000 single nucleotide polymorphisms (SNP) segregating in multiple populations. Using genome-wide association analyses, we identified multiple regions in the genome of the pathogen linked to increased asexual spore (pycnidia) production on wheat leaves. Characterization of the associated chromosomal regions showed that differences in virulence were most likely caused by non-synonymous substitutions in genes encoding cell wall-degrading enzymes and gene deletion polymorphisms of short secreted proteins. Second, we asked how chromosomal rearrangements can lead to either single gene or large segmental deletions. We used multiple fully assembled fungal genomes to quantify the extent of chromosomal rearrangements segregating within the species. We found that large clusters of transposable elements generated significant length polymorphism among homologous chromosomes. Extending this analysis to all resequenced isolates, we found that 530 genes of the core genome were missing in at least 20% of the isolates. Hence, Sexual reproduction and recombination in a population harboring significant chromosomal polymorphism enables the pathogen to rapidly gain or lose virulence loci in response to selection pressure imposed by the host.

## **Effector diversity seems to govern local adaptation of the rice blast fungus**

Jingjing Liao<sup>1</sup>, Hichuan Huang<sup>1</sup>, Isabelle Meusnier<sup>2</sup>, Aurelie Ducasse<sup>2</sup>, Francois Bonnot<sup>3</sup>, Elisabeth Fournier<sup>2</sup>, Pierre Gladieux<sup>2</sup>, Didier Tharreau<sup>3</sup>, Thomas Kroj<sup>2</sup>, Jean-Benoit Morel<sup>2</sup>

*1 College of Plant Protection, Yunnan Agricultural University, Kunming, China*

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

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

Scarce cases of disease durable resistance have been documented in plant/pathogen systems. Their thorough analysis may help to understand how durable resistance emerges and is maintained and how it can be exploited in a sustainable manner. We analyzed the case of the durable resistance of glutinous rice to blast disease caused by the fungus *Magnaporthe oryzae* in the Yuanyang terraces (Yunnan, China). Multi-year sampling of fungal isolates on glutinous rice and non-glutinous rice indicated that two populations of the blast fungus co-exist and are only rarely exchanged between these two rice hosts. Evaluation of the number of avirulence (Avr) effectors in the two *Magnaporthe oryzae* sub-populations demonstrated that isolates from glutinous rice possess particularly high numbers of Avr effectors. Moreover, aggressivity of these isolates on glutinous rice and non-glutinous rice varieties was correlated with the Avr effector content. Experiments with isogenic *M. oryzae* strains pinpoint one Avr effector that seem to play a key role in the local adaptation of the two blast sub-populations.





# Thursday 18<sup>th</sup> of February 2016

## Session 3

### R GENES & HOST TARGETS FOR RESISTANCE BREEDING AND ENGINEERING

Gregory MARTIN

#### **Using wild relatives of tomato to identify new components of the plant immune system**

Zhilong Bao, Patrick Boyle, Diane Dunham, Sarah Hind, Christine Kraus, Fanhong Meng, Kathy Munkvold, Susan Strickler, and Elise Viox, Gregory Martin

*Boyce Thompson Institute for Plant Research and Department of Plant Pathology and Plant-Microbe Biology, School of Integrative Plant Science, Cornell University, Ithaca, New York, USA*

The tomato immune system relies on at least three pattern recognition receptors (PRRs) to activate a first line of defense against *Pseudomonas syringae* pv. *tomato* (*Pst*), the causative agent of bacterial speck disease. Two of these PRRs, FLS2 and FLS3, act with BAK1 to recognize distinct portions of flagellin, while the other, Bti9, plays a role in detection of an unknown pathogen molecule. A major virulence mechanism of *Pst* is its type III secretion system which delivers ~30 effector proteins into the plant cell. One of these effectors, AvrPtoB, acts early in the infection process by interfering with FLS2, FLS3 and Bti9 functions. AvrPtoB is a 60 kd protein with multiple activities encoded by discrete structural domains. Some tomato varieties are immune to speck disease because they express the Pto kinase that interacts with AvrPtoB and acts in concert with the NLR protein Prf to activate effector-triggered immunity. Fen, another kinase related to Pto, also recognizes AvrPtoB but it is degraded by the E3 ligase activity encoded in the C-terminal domain of AvrPtoB. Protein structural biology and functional analyses have revealed the underlying basis for how immunity-associated kinases interact with various AvrPtoB domains. These insights have led to the development of a model for the evolutionary processes that have shaped the tomato-*Pst* interaction. Our current work is focused on integrating knowledge of AvrPtoB and various host kinases with what we are learning about natural variation in *Pst* recognition from characterization of wild tomato species.

## Matthieu JOOSTEN

### **Cf receptor-like proteins mount plant immunity in a complex with SOBIR1 and SERKs**

Aranka M. van der Burgh<sup>1</sup>, Jinbin Wu<sup>1</sup>, Tieme A. Helderma<sup>1</sup> and Guozhi Bi<sup>1,2</sup>, Matthieu Joosten<sup>1</sup>

*1 Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands*

*2 College of Horticulture, Northeast Agricultural University, Harbin 150030, China*

Receptor-like proteins (RLPs) are cell surface receptors that perceive microbial patterns and trigger plant immunity. RLPs lack an intracellular kinase domain and they constitutively interact with the receptor-like kinase (RLK) SOBIR1, which does have such a domain. Recently we showed that the tomato RLPs Cf-4 and Cf-9, providing resistance to the fungal pathogen *Cladosporium fulvum*, in complex with SOBIR1 recruit SERK3 (BAK1) and SERK1 upon their activation by the matching effectors, Avr4 and Avr9, respectively. Silencing of SERK gene expression revealed that the SERKs are essential for the activation of immunity. Thus, Cf-mediated immune signalling appears to be initiated by the formation of a tripartite receptor complex involving Cf-4, SOBIR1 and SERKs. Our current research is aimed at answering the following questions, and I will report on our progress at the meeting: Which domains of the various partners of the complex are essential for interaction? What is the structure of the Avr4/Cf-4/SOBIR1/SERK complex? We try to answer this question in collaboration with Prof. Dr. Jijie Chai (Tsinghua University, China). Does Cf-4 act as a receptor by directly binding the Avr4 protein? What is the role of SOBIR1 in downstream defence signalling by Cf-4? Is SOBIR1, in addition to being a binding scaffold for RLPs, also involved in phosphorylation processes in the cytoplasm? Which (cytoplasmic) signalling proteins are recruited downstream of the Avr4/Cf-4/SOBIR1/SERK complex? Can we further substantiate the presence of the Cf-4/SOBIR1/SERK tripartite complex

## Abbas MAQBOOL

### **Structural basis of effector recognition by a rice NLR immune receptor**

Abbas Maqbool<sup>1</sup>, Hiromasa Saitoh<sup>2</sup>, Marina Franceschetti<sup>1</sup>, Clare Stevenson<sup>1</sup>, Aiko Uemura<sup>2</sup>, Hiroyuki Kanzaki<sup>2</sup>, Sophien Kamoun<sup>3</sup>, Ryohei Terauchi<sup>2</sup>, Mark Banfield<sup>1</sup>

*1 John Innes Centre, United Kingdom*

*2 Iwate Biotechnology Research Center, Japan*

*3 The Sainsbury Laboratory, United Kingdom*

Plant pathogens deliver an arsenal of effector proteins into plant cells to promote infection. In response, plants have evolved intracellular immune receptors (NLRs) to recognize effector proteins. How this recognition operates at the molecular level remains largely unknown. In this study we investigated the structural basis of recognition of an effector, AVR-PikD from rice blast fungus, by an HMA domain integrated into the rice NLR Pikp. AVR-PikD binds a dimer of Pikp-HMA domain with nanomolar affinity. The crystal structure of AVR-PikD in complex with Pikp-HMA enabled us to design mutants in the effector protein in order to probe its activity in yeast, in vitro and in rice cultivar containing Pikp. Further, we also established an assay to study in planta responses in the model plant *Nicotiana benthamiana*. Together the data reveal the molecular details of a recognition event, which initiates a plant immune response and resistance to rice blast disease. Such studies can provide new strategies for crop protection and disease management.

## Identifying and Cloning of Novel Potato Immune Receptors Recognizing Apoplastic Effectors of *Phytophthora infestans*—Combined Effectoromics and Receptor Enrichment Sequencing

Xiao Lin<sup>1</sup>, Emmanouil Domazakis<sup>1</sup>, Katie Baker<sup>2</sup>, Doret Wouters<sup>1</sup>, Richard G. F. Visser<sup>1</sup>, Sophien Kamoun<sup>3</sup>, Ingo Hein<sup>2</sup> and Vivianne G. A. A. Vleeshouwers<sup>1</sup>

*1 Wageningen UR Plant Breeding, Droevendaalsesteeg 1, Wageningen 6708 PB, the Netherlands*

*2 The James Hutton Institute, Invergowrie, Dundee DD2 5DA, Scotland United Kingdom*

*3 The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, United Kingdom*

The potato (*Solanum tuberosum* L.) is one of the three most consumed crops worldwide. The most devastating disease of potato is late blight, which is caused by the oomycete *Phytophthora infestans*. Traditional disease resistance breeding that exploits cytoplasmic resistance genes (R genes) has been of limited success, as *P. infestans* has a remarkable capacity to rapidly adapt to resistant plants. Another, yet unexploited layer of immunity occurs at the surface of plant cells. This apoplastic immunity has generally a broader spectrum and is based on recognition of conserved proteins of pathogens. To obtain novel potato pattern recognition receptors (PRRs) that can recognize oomycete apoplastic effectors, we are deploying an effectoromics approach for high-throughput screening[1]. A variety of predicted oomycete apoplastic effectors are subjected to functional screens on almost 100 wild potato species. So far, all the cloned surface immune receptors are receptor-like kinase (RLK) or receptor-like protein (RLP). To accelerate gene mapping and cloning, we deployed an RLK/ RLP enrichment sequencing in the segregating populations[2]. All RLK/ RLP genes from the reference potato genome (DM) were predicted and used for designing a RNA bait library. The mapping parents and pools of responding and nonresponding F1 progenies were sequenced after RLK/ RLP gene enrichment. The pair end MiSeq reads were mapped to the DM genome and SNP calling was performed. Identified SNPs were selected for marker development. As a proof of concept, the ELR gene that confers response to INF1 was successfully mapped on the top of Chromosome 12. Currently, we are targeting a novel gene that triggers response to another apoplastic effector SCR74. Fine mapping of the SCR74 receptor is ongoing. Ultimately, we aim to pyramid diverse types of immune receptors to maximize the potential of generating a broader and potentially more durable resistance to *Phytophthora*.

## **The *Fusarium oxysporum* effector Six8 manipulates plant immunity through association with the transcriptional co-repressors TPL and TPR1**

FKK Gawehns<sup>1</sup>, M de Sainv, H Richter<sup>1</sup>, PM Houterman<sup>1</sup>, H Dekker<sup>2</sup>, D-J Valkenburg<sup>1</sup>, G Van den Ackervecken<sup>3</sup>, M Rep<sup>1</sup>, HA van den Burg<sup>1</sup> and Frank Takken<sup>1</sup>,

*1 University of Amsterdam, SILS, Molecular Plant Pathology, Sciencepark 904, 1098XH, Amsterdam, Netherlands*

*2 University of Amsterdam, SILS, mass-spectrometry-of-biomacromolecules, Sciencepark 904, 1098XH, Amsterdam, Netherlands*

*3 Utrecht University, Department of Biology, Plant-Microbe Interactions, Padualaan 8, 3584 CH Utrecht, the Netherlands*

*Fusarium oxysporum* f. sp. *lycopersici* (Fol) is the causal agent of tomato wilt disease. During infection the fungus secretes at least 50 proteins - including 30 enzymes and 14 candidate effectors - into the xylem sap. For the Six8 effector we found a potential host target; pull-down assays identified a member of the TOPLESS (TPL) family. The interaction of SIX8 with TPL and with a TPL homolog (TPR1-Topless Related 1) was confirmed in Y2H and BifC experiments. Silencing TPL/TPR1 in tomato resulted in increased resistance to Fol, classifying TPLs as genuine susceptibility genes. TPLs are co-repressors interacting with transcription factors that are involved in development, hormone signalling, and in "SNC1-mediated" defence mechanisms<sup>1</sup>. SNC1 is a NB-LRR resistance protein, providing a direct link between Six8 and host defence signalling. Transgenic *Arabidopsis* plants expressing SIX8 exhibit a temperature-dependent dwarf phenotype and show constitutive defence gene (PR1 and PR2) expression, suggesting a direct link to SNC1-mediated defences. *Arabidopsis* T-DNA insertion lines lacking SNC1 or components of the SNC1-defence pathway (i.e. *tpl*, *tpr1*, *tpr3*, *eds1*, *pad4*, and *NahG*) have been transformed with SIX8 to identify the pathways affected by Six8. Bioassays on SIX8-containing *Arabidopsis* plants using *P. syringae* and *H. parasitica* revealed that NB-LRR resistance proteins other than SNC1 are unaffected. This study identifies TPL as a genuine Six8 effector target. A possible mechanism of how Six8 triggers SNC1-mediated immune signalling will be presented, providing new leads to use effector-targets for disease resistance.

## **Targeted promoter editing for rice resistance to *Xanthomonas oryzae* pv. *oryzae* reveals alternative susceptibility pathways among SWEET-inducing TAL effectors**

Servane Blanvillain-Baufumé<sup>1</sup>, Maik Reschke<sup>2</sup>, Montserrat Solé<sup>2</sup>, Florence Auguy<sup>1</sup>, Hinda Doucoure<sup>1</sup>, Boris Szurek<sup>1</sup>, Donaldo Meynard<sup>3</sup>, Murielle Portefaix<sup>3</sup>, Sébastien Cunnac<sup>1</sup>, Emmanuel Guiderdoni<sup>3</sup>, Jens Boch<sup>2</sup>, Ralf Koebnik<sup>1</sup>

<sup>1</sup> UMR IPME, IRD-Cirad-UM, Montpellier, France

<sup>2</sup> Martin-Luther-Universität Halle-Wittenberg, Halle (Saale), Germany

<sup>3</sup> UMR AGAP, Cirad, Montpellier, France

Background: Bacterial leaf blight, caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo), is a major constraint for stable and sufficient rice production. To cause disease, bacteria inject DNA-binding proteins, called Transcription Activator-Like (TAL) effectors, into the plant cell where they manipulate the host transcriptome. In particular, induction of SWEET genes by TAL effectors is thought to provide the bacteria with favorable growth conditions, thereby allowing disease progression. Several TAL effectors from geographically distant Xoo isolates target OsSWEET14, which is thus considered as a pivotal susceptibility target of TAL effectors. Results: We generated an allele library of the OsSWEET14 promoter through transgenic expression of TALE-nuclease (TALEN) constructs in rice. Several transgenic plants carrying distinct edition events within the OsSWEET14 promoter, affecting three distinct TAL Effector Binding Elements (EBEs), were produced and the impact of homozygous mutations was assessed. Transgene-free plants stably edited in two of the TAL EBEs were resistant to bacterial strains relying on the corresponding TAL effector. Surprisingly, indels within the third TAL EBE preventing TAL effector-dependent OsSWEET14 expression did not lead to resistance to Xoo bacteria harboring the corresponding TAL effector. Moreover, Xoo-induced expression of clade-III SWEET genes was found to be dispensable for disease development. Conclusions: In contrast to the current dogma, our work demonstrates that knockout of a major susceptibility gene does not necessarily lead to resistance, as exemplified by the existence of additional target gene(s) for one of the OsSWEET14-inducing TAL effectors. Hence, TAL effector-mediated susceptibility of plants may result from induction of alternative susceptibility pathway(s).

## **R-gene domain architectures in flowering plants uncover host proteins targeted by the pathogens**

Panagiotis F. Sarris<sup>1</sup>, Volkan Cevik<sup>1</sup>, Gulay Dagdas<sup>1</sup>, Jonathan D. G. Jones<sup>1</sup>, Ksenia V Krasileva<sup>1, 2</sup>

*1 The Sainsbury Laboratory, Norwich Research Park, Norwich, United Kingdom*

*2 The Genome Analysis Centre, Norwich Research Park, Norwich, United Kingdom*

Intracellular plant immune receptors called NLRs are key initiators of plant defense responses. We have examined NLR domain architectures in 41 plant species from 14 families of flowering plants as well as algae and mosses and revealed that extraneous domains have repeatedly and frequently integrated into NLR proteins. We have identified conserved, recently formed and recurrent fusions between NLRs and other plant proteins suggesting continuous integration of domains as well as strong selection for particular fusions. Recent studies demonstrated that NLRs with non-canonical domain architectures deploy additional domains as baits for recognition of the pathogen-derived effector proteins. Availability of published effector interactome screens allowed us to overlay our analyses with predicted effector targets and revealed integration of known host targets in NLRs at variable rates across plant lineages. Some of the integrated domains are known to function in pathogen defense, such as RIN4, NPR1, while others originated from host proteins that may be deployed by pathogens to promote virulence and are prime candidates for functional analysis to engineer disease resistant plants.

## **The *Rx1\_ 'Gpa2* locus in potato: a molecular and genetic framework for engineering novel *NLR* genes**

Erik Slootweg, Jan Roosien, Erin Bakker, Rikus Pomp, Jaap Bakker, Aska Govere

*Laboratory of Nematology, Dept of Plant Sciences, Wageningen University, the Netherlands*

Plants are constantly exposed to a diverse array of pathogens and parasites that attempt to invade leaves, stems, or roots by various mechanisms. To sense foreign invaders, plants have evolved a cell autonomous immune system consisting of specific receptor-like proteins, including nucleotide binding domain and leucine-rich repeat containing proteins (NLRs), which confer host specific resistance upon recognition of pathogen elicitors. The close homologs *Gpa2* and *Rx1* confer resistance in potato to taxonomically unrelated pathogens: the cyst nematode *Globodera pallida* and Potato virus X (PVX), respectively. This provides us with a model system to study evolutionary and molecular aspects involved in pathogen recognition and NLR activation in plants. Our results demonstrate that complex NLR loci provide a genetic framework in which intergenic sequence exchange between homologous genes is allowed, but also point to functional constraints that act on the generation of effective novel NLR proteins. Sequence exchange results often in gain or loss of function phenotypes due to incompatibility between functional domains involved in regulating the molecular switch function of these proteins. However, functionality can be restored by modulating the sensitivity of the protein. This knowledge contributes to a better understanding of NLR evolution, but provides us also with a functional framework for engineering novel NLR genes based on gene shuffling and targeted mutagenesis.





# Friday 19<sup>th</sup> of February 2016

## Session 4

### PLANT PROTEINS AND PROCESSES TARGETED BY EFFECTORS

Regine KAHMANN

#### **The secreted effector repertoire of smut fungi**

*Max Planck Institute for Terrestrial Microbiology, Karl-von-Frisch-Str. 10, Marburg, Germany*

Smut fungi comprise a large group of biotrophic pathogens that infect cereal crops and wild grasses. The best studied member of this group, *Ustilago maydis*, infects maize and induces characteristic tumor formation and anthocyanin induction. During host colonization, *U. maydis* establishes an extended interaction zone with the plant in which fungal hyphae are encased by the host plasma membrane. Interaction with the plant is largely determined by protein effectors that are conventionally secreted and are induced only after plant colonization. A successful colonization requires active effector-mediated suppression of plant defense responses and host tissue reprogramming. Secreted effector proteins can either display their activity in the apoplast or translocate to host cells. While bacterial pathogens use Type III secretion systems for injecting effectors into plant cells, the molecular mechanism of effector delivery by eukaryotic plant pathogens remains elusive. In addition, we do not yet know what determines effector uptake by plant cells or retention in the apoplast. In my presentation I will report on the establishment of an uptake assay that is based on the ability of a bacterial biotin ligase, BirA, to biotinylate proteins in vivo that carry a small peptide tag (AviTag). In addition, I will focus on the regulation of effector genes and their functional analysis.

## **NAC transcription factors as susceptibility targets of the potato cyst nematode *Globodera pallida***

Sophie Mantelin<sup>1</sup>, Coke, M<sup>2</sup>, Wright K<sup>1</sup>, Thorpe P<sup>1,2</sup>, Cock P.J<sup>1</sup>, Smith A, Urwin P.E.<sup>2</sup> and Jones J.T<sup>1</sup>

<sup>1</sup> Cell and Molecular Sciences group, James Hutton Institute, Invergowrie, Dundee, DD2 5DA, United Kingdom

<sup>2</sup> Department of Plant Sciences, University of Leeds, Leeds, LS2 9JT, United Kingdom

During a compatible interaction, the potato cyst nematode *Globodera pallida* induces complex changes in its host, resulting in the formation of its feeding site, the syncytium. Effectors, which are mainly produced in the pharyngeal gland cells of the nematode and delivered to the plant cells through the stylet, are thought to be important in promoting establishment and maintenance of the syncytium. The completion of the *G. pallida* genome sequence (Cotton et al., 2014) has allowed identification of the full effector complement of this species (Thorpe et al., 2014). Most effectors are pioneers with no similarity to other sequences in databases. In order to shed light onto the biological function of such effectors, we have undertaken a large scale analysis of their subcellular localisation in planta using transient expression of fluorescent reporter fusions. In addition, many effectors were used as bait in yeast two-hybrid (Y2H) screens to identify potential host target proteins. Among the effectors investigated, only one localised to the endoplasmic reticulum. It represents a unique gene in *G. pallida*, which is specifically expressed in pre-parasitic and parasitic stage juveniles. Screening different potato Y2H libraries identified several transcription factors from the NAC family that showed variable strength of interaction and different subcellular localisation patterns in planta. Cell biological and chromatin binding analyses suggest that the effector stabilises the NAC proteins in planta. Overexpression of the effector in host plants seems to interfere with control of the plant defense response.

## **AN ADAPTOR KINASE CONFERS EXPANDED RECOGNITION SPECIFICITY TO A PLANT NLR**

Brice Roux<sup>1,2</sup>, Guoxun Wang<sup>3</sup>, Feng Feng<sup>3</sup>, Endrick Guy<sup>1,2</sup>, Lin Li<sup>4</sup>, Nannan Li<sup>4</sup>, Martine Lautier<sup>1,2,5</sup>, Marie-Françoise Jardinaud<sup>1,2</sup>, Matthieu Chabannes<sup>1,2</sup>, Matthieu Arlat<sup>1,2,5</sup>, She Chen<sup>4</sup>, Chaozu He<sup>6</sup>, Jian-Min Zhou<sup>3</sup> and L D. Noël<sup>1,2</sup>

1 INRA, Laboratoire des Interactions Plantes Micro-organismes (LIPM), UMR 441, Castanet-Tolosan, France

2 CNRS, Laboratoire des Interactions Plantes Micro-organismes (LIPM), UMR 2594, Castanet-Tolosan, France

3 State Key Laboratory of Plant Genomics, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, No. 1 West Beichen Road, Beijing 100101, China

4 National Institute of Biological Sciences, Beijing 102206, China

5 Université Paul Sabatier, Toulouse, France

6 Hainan University, Haikou, China

Effector proteins of pathogenic microbes utilize diverse biochemical activities to perturb cellular processes in host plants and animals, promoting parasitism. However, these biochemical activities can betray the pathogen by triggering host immunity when plants and animals carry cognate NOD-Like Receptors (NLRs) as a result of host-pathogen co-evolution. The *Xanthomonas campestris* effector protein AvrAC/XopAC inhibits plant immunity by uridylylating the Arabidopsis BIK1 kinase. Here, we show that AvrAC also uridylylates a related kinase, PBL2, which is required for resistance but dispensable for AvrAC virulence function in plants. PBL2 acts as a BIK1 decoy and is perceived by a stable pre-activation complex made of the pseudokinase RKS1 of the ZRK family and the NLR protein ZAR1. ZAR1 and the ZRK ZED1 were previously reported to confer recognition of an unrelated *Pseudomonas syringae* effector. Our results thus show how a plant ZRK adaptor kinase specifies and expands the recognition spectrum of an evolutionary-conserved NLR to compete in the arms-race against multiple pathogens.

---

## **Renier VAN DER HOORN**

### **Manipulation of Apoplastic Hydrolases by *Pseudomonas syringae***

Balakumaran Chandrasekar, Tram Ngoc Hong, Takayuki Shindo, Farnusch Kaschani, Daniela Sueldo, Renier Van Der Hoorn

Plant Chemetics Laboratory, Department of Plant Sciences, University of Oxford, OX1 3RB Oxford, United Kingdom

Plants respond to pathogen infection by secreting a battery of hydrolases that include proteases, lipases and glycosidases. These enzymes may contribute to the immune response by hydrolysing pathogen structures, and/or by releasing pathogen- or host-derived elicitors that amplify the immune response. We hypothesise that when living in the apoplast during infection, *P. syringae* suppresses the activity of these host hydrolases by secreting inhibitors. We develop and apply activity-based protein profiling to demonstrate the suppression of various apoplastic hydrolases during infection and are currently characterizing inhibitors of cysteine proteases, subtilases and beta-galactosidases. Depletion of the host hydrolase often increases bacterial growth, whilst inhibitor depletion reduces virulence, demonstrating that apoplast manipulation is important for virulence by *P. syringae*.

## **Plant histone H3 methyltransferases targeted by an aphid effector: a regulatory strategy for the suppression of Arabidopsis defences?**

Maëlle Jaouannet Rodriguez-Coloma PA<sup>1,2</sup>, Lenoir CJ<sup>1,2,3</sup>, Rivas s<sup>4,5</sup>, Jauneau A<sup>6</sup>, Pouzet C<sup>6</sup>, Escudero-Martinez C<sup>1,2,3</sup>, Thorpe P<sup>1,2</sup>, Nitsche A. and Bos JIB<sup>1,2,3</sup>

*1 Cell and Molecular Sciences; The James Hutton Institute; Dundee, United Kingdom*

*2 Dundee Effector Consortium; The James Hutton Institute; Dundee, United Kingdom*

*3 Division of Plant Sciences, College of Life Science, University of Dundee, Dundee, United Kingdom*

*4 INRA, UMR 441 Laboratoire des Interactions Plantes-Microorganismes, F-31326 Castanet-Tolosan, France*

*5 CNRS, UMR 2594 Laboratoire des Interactions Plantes-Microorganismes, F-31326 Castanet-Tolosan, France*

*6 CNRS, Plateforme Imagerie-Microscopie, Fédération de Recherche FR3450, 31326 Castanet-Tolosan, France*

Aphids, as other biotrophes, are able to suppress plant defences through the secretion of effectors<sup>3-6</sup>. Aphid effectors are produced in salivary glands and secreted with saliva into the plant tissues during probing and feeding<sup>3-7</sup>. Sets of predicted putative effectors have been recently identified<sup>8,9</sup> but only a limited number of these candidate have been characterized to date and have been implicated in promoting/decreasing virulence and activating/suppressing defences<sup>10</sup>. MpSecA has been identified in *Myzus persicae* saliva. According to my preliminary results, this effector plays a key role in the infestation success. We have showed that the silencing of the gene encoding MpSecA decreased the ability of *M. persicae* to infest *Arabidopsis*. Conversely, plants over-producing MpSecA are more susceptible to aphid infestation. We have recently demonstrated that MpSecA is interacting with two histone H3 arginine methyltransferases, AtPRMTA and AtPRMTB. We didn't observe any significant reduction of *M. persicae* fitness on single *AtprmtA/AtprmtB* knockout mutants. Interestingly, a double mutant *AtprmtA-AtprmtB* appears more resistant to aphid infestation suggesting that both MpSecA and its plant targets are essential to the aphid infestation success. Our fresh results support the hypothesis that aphid MpSecA promotes the host arginine-methyltransferase activity on histone H3 inducing the repression of plant gene expression. Indeed, I have shown that MpSecA induces chromatin over-compaction (Jaouannet and Bos, preliminary data). The functional characterisation of MpSecA are opening up an exciting area of research addresses that chromatin remodelling and/or histone post-translational modifications could act as important regulators of plant defences and thus facilitate the success of aphid infestation.

## **Subversion of autophagy by the Irish famine pathogen *Phytophthora infestans***

Yasin Dagdas<sup>1</sup>, Khaoula Belhaj<sup>1</sup>, Abbas Maqbool<sup>2</sup>, Angela Chaparro-Garcia<sup>1</sup>, Pooja Pandey<sup>4</sup>, Benjamin Petre<sup>1</sup>, Neftaly Cruz-Mireles<sup>1</sup>, Nadra Tabassum<sup>4</sup>, Richard K. Hughes<sup>2</sup>, Jan Sklenar<sup>1</sup>, Joe Win<sup>1</sup>, Frank Menke<sup>1</sup>, Kim Findlay<sup>3</sup>, Mark J. Banfield<sup>2</sup>, Sophien Kamoun<sup>1</sup> and Tolga O. Bozkurt<sup>1,4</sup>

*1 The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, United Kingdom*

*2 Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, United Kingdom*

*3 Department of Cell and Developmental Biology, John Innes Centre, Norwich Research Park, Norwich, NR4 7UH, United Kingdom*

*4 Imperial College London, Department of Life Sciences, London, United Kingdom*

Autophagy is a multifaceted membrane trafficking pathway involved in adaptation to cellular stress conditions such as starvation and pathogen infection. Activation of autophagy leads to formation of special vesicular structures called autophagosomes, which carry autophagic cargo to lysosomes or vacuoles for degradation. A form of autophagy, known as selective autophagy, can specifically degrade toxic substances such as invading pathogens. Selective autophagy functions through autophagy cargo receptors that confine the cargo within a special set of autophagosomes. Although the role of autophagy in antibacterial defense responses has been documented in animals, the role of autophagy in plant-microbe interactions is unclear and somewhat controversial. Here, we discovered that a secreted RXLR-WY type effector of *Phytophthora infestans*, named PexRD54, binds to the autophagy marker protein ATG8. We identified an ATG8 Interacting Motif (AIM) in PexRD54. Mutations in the AIM prevented both in vivo and in vitro PexRD54-ATG8 interactions. PexRD54 did not have a negative effect on autophagic flux and stimulated autophagosome formation. To investigate the biological function of PexRD54, we studied the autophagy cargo receptor Joka2, which also interacts with ATG8. Overexpression of Joka2 in planta limited *P. infestans* infection, suggesting a role for Joka2/ATG8 selective autophagy in response to oomycete infection. Remarkably PexRD54, but not the AIM mutant of PexRD54, was able to out-compete Joka2 for binding to ATG8 and restore full pathogen virulence. Our findings point to a model in which an RXLR-WY effector from *P. infestans* antagonizes a selective autophagy cargo receptor to enhance pathogen virulence.

## **The effector AWR5 from the plant pathogen *Ralstonia solanacearum* is an inhibitor of the TOR signalling pathway**

Crina Popa<sup>1, 2</sup>, Sergio Gil<sup>1</sup>, Laura Tatjer<sup>3</sup>, M. Tabuchi<sup>4</sup>, Joaquín Ariño<sup>3</sup>, Núria S. Coll<sup>1</sup> and Marc Valls<sup>1, 2</sup>

*1 Centre for Research in Agricultural Genomics (CSIC-IRTA-UAB-UB), Bellaterra, Catalonia, Spain*

*2 Genetics Department, Universitat de Barcelona, Barcelona, Catalonia, Spain*

*3 Institut de Biotecnologia i Biomedicina and Departament de Bioquímica i Biologia Molecular, Universitat Autònoma de Barcelona, Cerdanyola del Vallès, Catalonia, Spain*

*4 Laboratory of Applied Molecular and Cell Biology, Kagawa University, Kagawa, Japan*

Here, we show that the T3E AWR5 from the phytopathogen *Ralstonia solanacearum* is an inhibitor of TOR, a central regulator in eukaryotes that controls the switch between cell growth and stress responses in response to nutrient availability. Heterologous expression of AWR5 in yeast caused growth inhibition and autophagy induction coupled to massive transcriptomic changes, unmistakably reminiscent of TOR inhibition by rapamycin or by nitrogen starvation. Detailed genetic analysis of these phenotypes in yeast, including suppression of AWR5-induced toxicity by mutation of CDC55 and TPD3, encoding regulatory subunits of the PP2A phosphatase, indicated that AWR5 might exert its function by directly or indirectly inhibiting the TOR pathway upstream PP2A. We present evidence in planta that this T3E caused a reduction in TOR-regulated plant nitrate reductase activity and also that the bacterial growth inhibition caused by delivery of AWR5 into host cells was mediated by TOR. Our results suggest that TOR is a bona fide T3E target and validate yeast as a platform for T3E function characterisation.

## **Identification and functional analysis of a fungal effector suppressing apical dominance in maize**

Frank Drechsler<sup>1</sup>, Hassan Ghareeb<sup>2</sup>, Melissa Rohmich<sup>1</sup> and Jan Schirawski<sup>1,2</sup>

*1 RWTH Aachen University, Microbial Genetics, Institute of Applied Microbiology, Worringerweg 1, 52074 Aachen, Germany*

*2 Georg-August-University Göttingen, Molecular Biology of Plant-Microbe-Interactions, Schwann-Schleiden Centre, Julia-Lermontowa-Weg 2, 37077 Göttingen, Germany*

*Sporisorium reilianum* causes head smut on maize. Upon successful infection it induces severe morphological changes the inflorescences of its host. Male and female inflorescences are replaced by fungal spores or produce leafy structures. Additionally, outgrowth of subapical ears is induced that also contain fungal spores. Targeted gene deletion experiments identified the effector SUPPRESSOR OF APICAL DOMINANCE1 (SAD1) of *S. reilianum* as responsible for subapical ear development. Expression of a SAD1-GFP fusion protein revealed its secretion from fungal hyphae in leaves and ears. Transgenic *Arabidopsis thaliana* plants expressing GFP-SAD1 produced more branches than GFP-expressing control plants. In *A. thaliana*, GFP-SAD1 shows cytoplasmic and nuclear localization. We analyze the functional importance of SAD1 localization by forcing SAD1 to different plant cell compartments. In maize, SAD1 is able to interact with a great number of plant proteins. One of the strongest interactors of SAD1 is RGLG2 that is known to localize both to the cytoplasm and the nucleus and to be involved in apical dominance. We sequenced the RNA of ears infected with either *S. reilianum* wild-type strains or strains lacking SAD1. We will present data on deregulated genes involved in biosynthesis or transport of sugars or hormones, light perception, flower development, or genes known to be regulated by RGLG2. The result will lead to a basic understanding of how fungal effectors can manipulate plant development and be of possible use in the generation of plants with an increased number of flowers or a higher yield of seeds





# **ABSTRACTS OF POSTER PRESENTATIONS**



## **Specificity of recognition to *P. infestans* AVR2 effector family mediated by *Solanum* R gene families**

Carolina Aguilera-Galvez, Hendrix Rietman, Emmanouil Domazakis, Xiao Lin, Doret Wouters, Gerard Bijsterbosch, Richard G.F. Visser, Vivianne G.A.A. Vleeshouwers.

Wageningen UR Plant Breeding, Wageningen University and Research Center, The Netherlands

Late blight, caused by the oomycete *Phytophthora infestans* is nowadays the most devastating disease for potato crops. A common strategy to control the disease is the introgression of resistance (*R*) genes in potato cultivars. However, single-dominant host *R* genes have rapidly been defeated, due the genome plasticity of *P. infestans*. Nowadays, simultaneous deployment of multiple broad spectrum *R* genes is advised. Still, for educated *R* gene management, knowledge of the corresponding avirulence (*Avr*) genes is essential. In this study, we focus on AVR2, which is recognized by the R2 protein. Studies in the *Phytophthora* genome have revealed that *Avr2* belongs to a large, highly diverse gene family. Effector screens are being performed with *Avr2* family members in a wide collection of wild *Solanum* section *Petota* species. Preliminary data show the various AVR2 family members can cause cell death in a diversity of *Solanum* species that do not carry R2. This suggests that the recognition of AVR2 family members mediated by at least two different *Solanum* *R* gene families. We aim to isolate and functionally characterize *R* proteins and targets that are involved in recognition of AVR2 family effectors. Ultimately, we aim to exploit and pyramid those *R* genes and achieve a more broad-spectrum resistance to multiple members of the AVR2 family of *P. infestans*.

---

## **P2 : Philip ALBERS**

## **HopZ1a targets a remorin protein implicated in membrane-associated defence signalling**

Ph. Albers<sup>1</sup>, S. Üstün<sup>1</sup> and F. Börnke<sup>1,2</sup>

<sup>1</sup> Leibniz-Institute for Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany

<sup>2</sup> Institute for Biochemistry and Biology, University of Potsdam, Germany

HopZ1a, a member of the YopJ superfamily from *Pseudomonas syringae*, was shown to display acetyltransferase activity towards tubulin leading to the inhibition of secretion during defense responses. To identify new HopZ1a targets we initiated a yeast-two-hybrid (Y2H) screen and identified a remorin as interactor of HopZ1a which we named HopZ1a-interacting-protein 1 (HIR1). To characterize a role of HIR1 in plant immunity, we performed additional Y2H screens and identified PBS1, a protein kinase involved in plant defence, and SINA4, an E3 ubiquitin ligase as part of a putative HIR1 interactome. Using split-YFP, we confirmed the interaction of HopZ1a and HIR1, as well as HIR1 and PBS1, with both complexes associating at the plasma membrane. Cell biological approaches revealed that upon flg22 treatment HIR1 shifts into punctuated structures at the plasma membrane resembling lipid rafts. Furthermore, preliminary results indicate a role of HIR1 as a positive regulator of PTI, as PTI marker gene expression is increased in plants overexpressing HIR1 and ROS production is affected in plants silenced for HIR1. In summary, our findings support the hypothesis that HIR1 might act in a complex together with immune kinase PBS1 during PTI and hence is targeted by HopZ1a to manipulate membrane-associated defence responses.

### P3 : Nuno Felipe ALMEIDA

## **The pursuit of effector targets in *Lathyrus cicera* rust resistance QTLs**

Nuno Felipe Almeida<sup>1</sup>, Diego Rubiales<sup>2</sup>, Maria Carlota Vaz Patto<sup>1</sup>

*1 Instituto de Tecnologia Química e Biológica António Xavier, Universidade Nova de Lisboa, Apartado 127, 2781-901 Oeiras, Portugal*

*2 Institute for Sustainable Agriculture, CSIC, Alameda del Obispo s/n, Apdo 4084, 14080 Cordoba, Spain*

Rusts are among plant pathogens with broad host range, causing dramatic losses in various crops such as grain legumes. As biotrophic fungi, there is a requirement to sustain infected host cells alive for their development, increasing the need for an efficient cross-talk between plant host and pathogen. In order to manipulate the host's cell structure and function for effective colonization, rust pathogens secrete specific proteins, called effectors. Despite playing a key role in disease biology, the effectors repertoire associated with rust infection remains unclear. RNA-Seq analysis of pea rust (*Uromyces pisi*) / chickling vetch (*Lathyrus cicera*) interaction allowed the identification and quantification of expressed sequences potentially involved in plant resistance. Potential effectors involved in this plant pathogen interaction were also identified. Furthermore, QTL for pea rust resistance were mapped in chickling vetch using a RIL population resulting from the cross of rust resistance contrasting accessions. By integrating the information on the candidate genes underlying the rust resistance QTLs and the potential rust effectors detected by RNA-Seq, we searched for potential effector targets located in those regions. Results on this *in silico* detection of effector candidate targets underneath *Lathyrus cicera* rust resistance QTLs will be discussed.

-----

### P4 : Lander BAUTERS

## **Chorismate mutase and isochorismatase, two parasitism proteins of the nematode *Hirschmanniella oryzae*, increase susceptibility of rice to nematode infection**

Lander Bauters, Kyndt T.<sup>1</sup>, Haeck A.<sup>2</sup>, Demeestere K.<sup>2</sup>, De Meyer T.<sup>3</sup>, Gheysen G.<sup>1</sup>

*1 Department of Molecular Biotechnology, Ghent University, Ghent, Belgium*

*2 Department of Sustainable Organic Chemistry and Technology, Ghent University, Ghent, Belgium*

*3 Department of Mathematical Modelling, Statistics and Bio-informatics, Ghent University, Ghent, Belgium*

*Hirschmanniella oryzae*, a plant-parasitic migratory nematode with a world-wide distribution, is a devastating plague in flooded rice-ecosystems. Transcriptome data indicated the presence of chorismate mutase, an effector previously reported in plant-parasitic nematodes and fungi, and isochorismatase, which was not reported in nematodes before. Activity tests showed that both proteins are active in vitro. Rice overexpression lines were more susceptible to nematode infection. Although both proteins were thought to have an effect on the salicylic acid biosynthesis pathway in plants, no clear differences in hormone balances were observed. Preliminary results indicate that the reduced resistance is probably due to a shift in secondary metabolism. Transcriptome analysis of the overexpression lines revealed a general down-regulation of genes involved in phenylpropanoid and terpenoid metabolism. Histochemical staining showed a reduced terpenoid content in roots of overexpression lines. Further analyses are needed to confirm that a shift in secondary metabolism is responsible for the higher susceptibility, rather than a change in hormonal balance. These results indicate that both chorismate mutase and isochorismatase are used by the nematode to increase susceptibility of the host.

## **Identification of *Ustilago maydis* Effectors Targeting Hormonal Signaling**

Janos Bindics, Alexandra Stirnberg, Simon Uhse, Daniel Reumann, Laura Baggaley and Armin Djamei

Gregor Mendel Institute of Molecular Plant Biology GmbH, Dr. Bohr-Gasse 3, 1030 Vienna, Austria

*Ustilago maydis* is a biotrophic fungal pathogen of maize. During infection the penetrating hypha delivers high number of effector molecules into the host plant body in order to suppress defense mechanisms and to alter the host's metabolism on the pathogen's benefit. Despite the extensive research on this complex process our understanding is far from complete. Global transcriptome profiling of infected maize plants revealed prominent changes in the expression of numerous genes. These changes affect at least 3 hormone signaling pathways: **i)** jasmonic acid, **ii)** auxin and **iii)** gibberellic acid (Doehlemann *et al.* 2008). Moreover, *U. maydis* has been shown to be able to synthesize indole-3-acetic acid (auxin), which also contributes to the elevated auxin levels of the infected tissues (Reineke *et al.* 2008). Additionally *U. maydis* is able to sense and degrade salicylic acid, the central regulator of plant systemic defense reactions (Rabe *et al.* 2013). These pieces of evidence suggest that *U. maydis* has evolved several mechanisms, which target numerous hormonal signaling pathways of its host plant and they might play an important role in the infection process. Therefore, we aim to establish screens to test for hormonal signaling function of our effector candidate library (~280 genes). Here we present the progress of our screen, which led to the identification of a new group of effector molecules.

---

## **P6 : Eran BOSIS**

## **Identification of New *Xanthomonas* Type III Effectors by a Bioinformatic Approach**

Eran Bosis<sup>1,2</sup> and Guido Sessa<sup>2</sup>

1 Department of Biotechnology Engineering, ORT Braude College, Karmiel, Israel

2 Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Israel.

Bacteria belonging to the genus *Xanthomonas* can cause severe diseases in a wide variety of plant species. Type III effectors (T3Es), injected into the host cells by the type III secretion system (T3SS), play an important role in the pathogenicity and host specificity of *Xanthomonas* species. Significant efforts were made in recent years to identify the repertoire of *Xanthomonas* T3Es, resulting in the identification of more than 50 different effector families. In this work, we applied a bioinformatic approach to identify new *Xanthomonas* T3Es. First, we analyzed the amino acid frequency of the N-terminal residues of known *Xanthomonas* T3Es. We found that most amino acids were differentially represented in the N-terminal residues of T3Es. Next, we discovered that the N-terminal residues of T3Es were less likely to assume an ordered secondary structure. Finally, we found that known *Xanthomonas* T3Es exhibited very low homology to proteins in *Xanthomonas* strains lacking a T3SS. We combined these features using a machine-learning approach. We screened the genomes of 20 *Xanthomonas* strains encoding a T3SS and identified new putative T3Es in each of these genomes. Furthermore, by combining our model with detailed investigation of the translation initiation sites, we were able to identify many T3Es that their translation initiation sites should be reconsidered. Identifying the entire repertoire of T3Es would help us to better understand the mechanisms of bacterial virulence and host defense. The approach presented here is not limited to *Xanthomonas* and should be applicable for the identification of virulence factors in other phytopathogens.

## **Lectin receptor kinases in microbial recognition and plant immunity**

Natalie Verbeek<sup>1</sup>, Yan Wang<sup>1</sup>, Klaas Bouwmeester<sup>1,2</sup>

*1 Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands*

*2 Plant-Microbe Interactions, Utrecht University, Utrecht, The Netherlands*

Membrane-bound receptors play crucial roles as sentinels of plant immunity against a large variety of invading microbes. One class of receptors known to be involved in self/non-self-surveillance and plant resistance comprises the L-type lectin receptor kinases (LecRKs). In recent years, we reported that LecRKs function in resistance to *Phytophthora* pathogens in *Arabidopsis* (Wang et al., 2014), and showed that clade IX *N. benthamiana* and tomato LecRKs play similar roles in resistance as their *Arabidopsis* homologues (Wang et al., 2015), suggesting conserved functions across different plant families. By using phylogenomics approaches, we obtained more insight in the evolutionary history of LecRKs across plant families (Hofberger et al., 2015). This revealed that LecRKs are diverse and wide-spread in plants, and this justifies exploitation of LecRKs in crop resistance breeding. Various *Arabidopsis* LecRKs were as well found to play crucial roles in resistance to the pathogenic bacterium *Pseudomonas syringae*. This raised the question whether LecRKs play as well a role in interactions with related non-pathogenic microorganisms that are known to induce plant growth promotion and systemic resistance. Hence, we assayed *Arabidopsis* LecRK mutants for their response upon inoculation with the rhizobacterium *Pseudomonas fluorescens*. Results show that several LecRKs mutants are altered in their behaviour, suggesting that LecRKs are needed for rhizobacterium recognition and/or subsequent elicitation of plant growth promotion and resistance.

## **Understanding pea resistance mechanisms in response to *Fusarium oxysporum* through proteomic analysis**

María Angeles Castillejo, Moustafa Bani, Diego Rubiales

*Institute for Sustainable Agriculture, CSIC, 4084, 14080 Córdoba, Spain*

*Fusarium oxysporum* f. sp. pisi (Fop) is an important and destructive pathogen affecting pea crop throughout the world. To study the molecular basis of resistance to Fop we have used a proteomic approach (2-DE/MSMS analysis) with the aim of identify proteins of resistance. For such purpose the root proteome of three pea genotypes showing different levels of resistance to Fop race 2 were studied. Statistical analysis revealed 132 differential proteins, of which 53 were identified and functionally categorized: carbohydrate and energy metabolism (21%), nucleotides and aminoacid metabolism (7%), signal transduction (8%), folding (8%), redox (13%), defense (9%), biosynthetic process (24%) and transcription/translation (4%). We have focused in signal transduction proteins: 14-3-3-like protein and intracellular chloride channel. In plants, 14-3-3s bind many effectors that are secreted by pathogens, acting as receptors of fungal toxins in plant–fungus encounter. We identified both proteins increased in the resistant genotype in response to infection, fact that may be implicated in wilt suppression. Results obtained suggest that the most susceptible genotypes increased levels of enzymes involved in the production of reducing power which could then be used as cofactor for enzymes of redox reactions. This is in concordance with the fact that a ROS burst occurred in these genotypes, as well as an increase of PR proteins. However the ROS generation failed to prevent fungal colonization or reproduction within the xylem tissues. Conversely, in the resistant genotype proteins responsible to induce changes in the membrane and cell wall composition related to reinforcement were identified.



## P9: James COCKRAM

### **EffectaWheat: An Effector- and Genomics-Assisted Pipeline for *Necrotrophic Pathogen* Resistance Breeding in Wheat**

Lorenz Hartl<sup>2</sup>, Sarah Holdgate<sup>1</sup>, Lise Jørgensen<sup>3</sup>, Annemarie Justesen<sup>3</sup>, Volker Mohler<sup>2</sup>, Morten Lillemo<sup>4</sup>, Ian Mackay<sup>1</sup>, Caroline Moffat<sup>5</sup>, Richard Oliver<sup>5</sup>, Kar-Chun Tan<sup>5</sup>, Pao Theen See<sup>5</sup>, Judith Turner<sup>6</sup>, James Cockram<sup>1</sup>

*1 NIAB, Huntingdon Road, Cambridge, CB3 0LE, United Kingdom*

*2 LFL, Am Gereuth 6, 85354 Freising, Germany*

*3 AARHUS, Forsøgsvej 1, DK-4200, Slagelse, Denmark*

*4 NMBU, Vollveien 3, 1430 Ås, Norway*

*5 CCDM, Curtin University, WA, Australia*

*6 FERA, National Agrifood Innovation Campus, Sand Hutton, York, YO41 1LZ, United Kingdom*

EffectaWheat is a recently funded project under the ERA-CAPS call. It is a partnership of six complementary institutes from the UK (NIAB, FERA), Germany (LFL), Denmark (AARHUS), Norway (NMBU) and Australia (CCDM). The partners form a multidisciplinary team focusing on the economically important wheat leaf spot group (LSG) of necrotrophic pathogens: *Parastagonospora nodorum* (Pn, cause of Septoria nodorum blotch; SNB), *Zymoseptoria tritici* (Zt, Septoria tritici blotch; STB) and *Pyrenophora tritici-repentis* (Ptr, tan spot; TS). Recently available resources in wheat, including high-resolution genetic mapping populations and high-density genotyping, will be combined with emerging tools and approaches in the pathogens, such as pathogen genome resequencing, and identification of pathogen-produced molecules that mediate infection (termed, 'effectors'). These approaches will complement the project partners' expertise in pathogen diagnostics, virulence assessment and field pathotesting across all partner countries. These combine to deliver a genomics- and effector-based pipeline for the genetic dissection of LSG host-pathogen interactions in Europe. The unique positions of partners at the interface between crop research and translation ensure effective dissemination of project outputs to European agri-industry. This approach has been successfully implemented by CCDM for SNB and TS in Australia. This project will extend the approach to Europe.

## **StERF, an Ethylene Response Factor involved in potato defence response to PVY**

Hazel McLellan<sup>2</sup>, Ana Lazar<sup>1</sup>, David Dobnik<sup>1</sup>, Neža Turnšek<sup>1</sup>, Špela Baebler<sup>1</sup>, Paul Birch<sup>2,3</sup> and Kristina Gruden<sup>1</sup>, Anna Coll<sup>1</sup>

*1 Department of Biotechnology and Systems Biology, National Institute of Biology, Ljubljana, Slovenia*

*2 Division of Plant Sciences, University of Dundee (at James Hutton Institute), Invergowrie, Dundee, United Kingdom*

*3 Cell and Molecular Sciences, The James Hutton Institute, University of Dundee, Invergowrie, Dundee, United Kingdom*

Potato (*Solanum tuberosum* L.) is the world's most widely grown tuber crop and potato virus Y (PVY) is one of the major potato pathogen causing severe crop loss in different areas worldwide. To better understand the potato defence response against PVY we studied the role of ethylene response factor (ERF) genes from group IX since they have been related to plant defence response and defined as important elements on hormone crosstalk. Potato ERF-IX genes were identified and classified in this study. Among them, StERF was selected for further analyses based on previous transcriptomics experiments performed in our group (1). Expression patterns of the gene in hypersensitive resistance (HR) potato cultivar infected with PVY pointed to its importance as a signalling component in potato defence response. Using virus-induced gene silencing (VIGS) we demonstrated that PVY systemic spread is delayed in StERF silenced plants. We further examined the potential hormonal signalling involved in the expression of StERF and demonstrated that our gene integrates several signalling pathways. By means of Y2H the selected gene has been subjected to screening a potato cDNA library in order to identify their interaction partners. Getting more insights into the regulation of the gene, localisation studies showed that StERF strongly accumulated in cell nucleus after PVY infection. Taken together our results suggested the importance of StERF in potato-PVY interaction. Therefore the data contributes to better understand the complex network of plant defence signalling pathways.

## ***Magnaporthe oryzae* effectors AVR-Pia and AVR1-CO39 Reveal Structural Homology**

Karine de Guillen<sup>1</sup>, Diana Ortiz-Vallejo<sup>2</sup>, Jérôme Gracy<sup>1</sup>, Elisabeth Fournier<sup>2</sup>, Thomas Kroj<sup>2</sup> and André Padilla<sup>1</sup>

*1 CBS Centre de Biochimie Structurale, INSERM U1054, CNRS UMR5048, University of Montpellier, Montpellier, France*

*2 BGPI Biologie et Génétique des Interactions Plantes-Pathogènes, INRA- CIRAD - Montpellier SupAgro, Montpellier, France*

Plant pathogen genomes co-evolve with their host genomes to overcome the plant resistance mechanisms through generation of a variety of isolates. The causal agent of rice blast, *Magnaporthe oryzae*, the major Rice pathogen, is responsible of economically significant crop losses. During the infection stage the fungus secretes small proteins acting as virulent factors, called effectors, some of them being translocated inside host cells. Among them, avirulent effectors are recognized by the plant immune system through cytoplasmic receptor proteins that activates “effector triggered immunity” mediating the resistance answer. Here, we solved the NMR structures of two such effectors of *M.oryzae*: AVR-Pia and AVR1-CO39. Structurally, they share a common three-dimensional architecture, also found in *M. oryzae* AVR-Piz-t effector and *Pyrenophora tritici repens* toxin ToxB, pathogenic for wheat. Sequence comparison search of fungi protein databases based in the 3D structures and sequence alignments, revealed new possible members of this sub-class of plant pathogen effectors.

## **Exploring the role of Rbp-1 in *Globodera pallida* parasitism**

Amalia Diaz Granados, Hein Overmars, Roel Ariaans, Casper van Schaik, Eric Slootweg, Jaap Bakker, Geert Smant, Aska Govere

*Department of Nematology, Wageningen University, The Netherlands*

Persistent nematode infections are a major threat to important food crops. These round worms manipulate plant cell morphology and physiology to establish sophisticated feeding structures. Modifications to plant cells are largely attributed to the activity of nematode secreted effectors. SPRYSECs are a remarkably expanded family of effectors identified initially in the potato cyst nematode *Globodera rostochiensis*. In the sibling species *Globodera pallida*, a SPRYSEC subfamily is present called RBP-1. Although some members are specifically recognized by the Gpa2 resistance gene from potato, their role in nematode virulence is still unknown. To address this question, we performed a Y2H screening of a nematode-infected susceptible potato library to identify host targets involved in nematode parasitism. This yielded a number of interacting candidates involved in post-translational modification in plants. We have independently confirmed that two ligases involved in post-translational modification can interact with both virulent and avirulent variants of Rbp-1 in yeast. A localization study also shows that the candidate interactors localize to the nucleus, which allows interaction with Rbp-1 as it shows a nucleocytoplasmic localization pattern. Upon co-expression of the interactors, a shift towards to nucleus was observed for RBP-1 suggesting that they reside indeed in the same complex. Furthermore, upon silencing of the corresponding ligase genes in *A. thaliana*, we observed significant differences in the amount of nematodes present in the roots of nematode infected plants, indicating their importance for nematode parasitism. These candidate interactors of Rbp-1 suggest that the intrinsic role of the effector is carried out through manipulation of the plant post-translational modification machinery. Our findings suggest that nematodes are able to use this repertoire of effectors to control different aspects of the plant cell to establish a feeding site. Therefore our results may provide further insight into the basis of virulence of nematodes in plants.

## **Mapping the wheat Snn1 locus conferring sensitivity to the *Parastagonospora nodorum* necrotrophic effector SnTox1 using an eight founder multi-parent advanced generation intercross population**

Rowena Downie<sup>1</sup>, James Cockram<sup>1</sup>, Alice Scuderi<sup>1,2</sup>, Toby Barber<sup>1</sup>, Eiko Furuki<sup>3</sup>, Keith A. Gardner<sup>1</sup>, Nick Gosman<sup>1</sup>, Radoslaw Kowalczyk<sup>1,4</sup>, Huyen P. Phan<sup>3</sup>, Gemma A. Rose<sup>1</sup>, Kar-Chun Tan<sup>3</sup>, Richard P. Oliver<sup>3</sup>, Ian J. Mackay<sup>1</sup>

*1 John Bingham Laboratory, National Institute of Agricultural Botany (NIAB), Huntington Road, Cambridge, CB3 0LE, United Kingdom*

*2 Department of Drug Science and Products for Health, University of Messina, Messina, Sicily, 98122, Italy*

*3 Centre for Crop Disease Management, Curtin University, WA6845, Australia*

*4 Faculty of Life Sciences, University of Manchester, Manchester, M13 9PL, United Kingdom*

The necrotrophic fungus *Parastagonospora nodorum* is an important pathogen of one of the world's most economically important cereal crops, wheat (*Triticum aestivum* L.). *P. nodorum* produces necrotrophic protein effectors that mediate host cell death, providing nutrients for continuation of the infection process. The recent discovery of pathogen effectors has revolutionised disease resistance breeding for necrotrophic diseases in crop species, allowing often complex genetic resistance mechanisms to be broken down into constituent parts. To date, three effectors have been identified in *P. nodorum*. Here we use the effector, SnTox1, to screen 642 progeny from an eight parent multi-parent advanced generation inter-cross (MAGIC) population, genotyped with a 90,000 feature single nucleotide polymorphism array. The MAGIC founders showed a range of sensitivity to SnTox1, with transgressive segregation evident in the progeny. SnTox1 sensitivity showed high heritability, with quantitative trait locus analyses fine-mapping the Snn1 locus to the short arm of chromosome 1B. In addition, a previously undescribed SnTox1 sensitivity locus was identified on the long arm of chromosome 5A, termed here QSnn.niab-5A.1. The peak SNP for the Snn1 locus was converted to the KASP genotyping platform, providing breeders and researchers a simple and cheap diagnostic marker for allelic state at Snn1.

## **Investigation of *Heterodera schachtii* transcriptome to identify putative effectors**

Elashry A<sup>1</sup>, Habash S<sup>1</sup>, Ahmadinejad N<sup>2</sup>, Schoof H<sup>2</sup>, Vijayapalani Paramasivan<sup>3</sup>, Thomas Maier<sup>3</sup>, Thomas Baum<sup>3</sup>, Grundler F.M.W<sup>1</sup>

*1 INRES Molecular Phytomedicine, University Bonn, Karlrobert-Kreiten-Str.13, 53115 Bonn, Germany*

*2 INRES Crop Bioinformatics, University Bonn, Katzenburgweg2, 53115 Bonn, Germany*

*3 Department of Plant Pathology, Iowa State University, Ames, Iowa 50011, USA*

The beet cyst nematodes (BCN) *Heterodera schachtii* (Hs) depend on a set of secretory proteins (effectors) for the induction and maintenance of their syncytial feeding sites. In order to understand the relationship between Hs and its host, the identification of Hs effectors is a crucial step. We sequenced pre- and post-infective developmental stages using Next Generation Sequencing (NGS) forming transcriptome assembly. The resulted transcriptome was translated and analysed structurally to identify the Putative Secretory Proteins (PSPs). This procedure resulted in the identification of nearly 1000 PSPs. However, we needed to further investigate the PSPs dataset determining which of the PSPs can be identified as putative effector. Normally, putative effector is upregulated in one or more of the post infective stages and having its expression localised to the esophageal gland. We compared the whole transcriptome with published *Gloptera pallida* sequences that were upregulated in one or more developmental stages and esophageal gland sequences. Our analysis resulted in the identification of 14 PSPs. In order to validate the resulted PSP subset as a putative effectors, we analysed them by qPCR and in situ hybridization. Sequences that have shown upregulation and specific localization in esophageal gland were considered putative effectors. Knocking down the identified putative effectors using RNAi has shown significant effect on the level of parasitism. The decrease of the parasitism level by manipulating the target putative effectors help validating them and may reflect an applicable way to minimize the economical losses caused by BCN infection.

**P15: Maria Raffaella ERCOLANO**

## **Comparative genomic approaches for investigating *Solanaceae* defence system**

Di Donato A., Andolfo G., Ercolano M.R.

*Department of Agriculture, University of Naples "Federico II", Via Università 100, 80055 Portici, Italy*

Multiple omics approaches lead to new insights into plant-pathogen interactions, owing to the availability of high throughput biological data and computational tools able to extract important meanings. A Plant Resistance Genes database, (<http://www.prgdb.org>) was established in order to provide a comprehensive overview of plant resistance genes (R-genes). It is community database for plant scientists who could in turn contribute to this public resource through a WIKI-like system. The inferred cross-link between genomic and phenotypic information allows to access to a large body of information to find answers to several biological questions. Our database offers a range of querying and mining tools, including *Solanaceae* metaspecies section. As proof of concept, tomato, potato and pepper and Eggplant putative pathogen recognition genes were annotated with our specific predictor and characterized with respect to structural diversity, phylogenetic relationships and chromosomal distribution. A first genome-wide comparative analysis of candidate pathogen recognition genes in the *Solanaceae* was conducted underlying mechanisms of molecular adaptive selection at *Solanum* R loci. Analysis of main R-gene clusters allowed to reconstruct their evolution history.

---

**P16: Lennart ESCHEN LIPPOLD**

## **Bacterial effector-mediated suppression of PAMP-induced defence signaling**

Lennart Eschen Lippold, Dierk Scheel, Justin Lee

*Leibniz Institute of Plant Biochemistry, Weinberg 3, Halle, D-06120, Germany*

During co-evolution with microorganisms, plants evolved membrane-resident receptors to specifically sense the presence of attacking pathogens by recognition of pathogen-associated molecular patterns (PAMPs). These are highly conserved motifs present in microbial molecules essential for their lifestyle. Prominent examples are flg22 and elf18, two short amino acid motifs found in the bacterial proteins, flagellin and elongation factor Tu, respectively. Upon ligand-binding, receptors are activated and, within minutes, diverse defence signalling responses are initiated, including ion fluxes across the plasma membrane, calcium influx into the cytosol, generation of reactive oxygen species and activation of mitogen-activated protein kinases (MAPKs). MAPK-mediated phosphorylation of diverse substrate proteins represents an important mechanism to regulate protein abundance and activity. To intercept establishment of a proper defence response, pathogens evolved effector molecules which are directly delivered to the host cells, targeting specific components of the defence signalling machinery. Several bacterial effectors are known to interfere with MAPK activity. Among them, HopF2, was shown to bind MKK5 and possibly other MKKs to ADP-ribosylate their C-termini, blocking phosphorylation activity. Another effector, HopAI1, has phosphothreonine lyase activity and targets MPK3, MPK4 and MPK6 to inactivate them by removing the phosphate group in the TEY motif. We identified an effector specifically suppressing PAMP-induced MPK4 and MPK11 activation. Its protease activity is required for the suppression, although both kinases themselves are not degraded. As a result, several defence-related genes are affected in their expression, correlating with enhanced susceptibility against *Pseudomonas syringae* and *Botrytis cinerea*.

## **The evolution and diversification of novel biosynthetic function/s in effectors: a basis for specific pathogen-informed drug design?**

Sebastian Eves-van den Akker, Catherine J. Lilley<sup>3</sup>, Laura M. Jones<sup>3</sup>, Hazijah B. Yusup<sup>3</sup>, Abbas Maqbool<sup>2</sup>, Mark Banfield<sup>2</sup>, Paul Birch<sup>1</sup>, John T. Jones<sup>4,5</sup> and Peter E. Urwin<sup>3</sup>

*1 School of Life Sciences, University of Dundee, Dundee, DD1 5EH, United Kingdom*

*2 Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich NR4 7UH, United Kingdom*

*3 Centre for Plant Sciences, University of Leeds, Leeds, LS2 9JT, United Kingdom*

*4 Cell and Molecular Sciences Group, James Hutton Institute, Invergowrie, Dundee, DD2 5DA, United Kingdom*

*5 School of Biology, University of St Andrews, North Haugh, St Andrews, KY16 9TZ, United Kingdom*

Many effectors in plant-parasitic nematodes are part of large multi-gene families, yet, the evolutionary pathway giving rise to effectors is often unclear. In exception to this are the glutathione synthetase(GS)-like effectors. A clear, two-step, evolutionary process gave rise to the present three clade family where: the “basal” gene of Clade 1 appears to be common to all nematodes; the “intestinal” genes of Clade 2 appear to be common to endoparasites; while the effector genes of Clade 3 appear to be specific to a single lineage of plant-parasitic nematodes. Despite the fact that each expansion witnessed a cumulative 10 fold reduction in glutathione synthetic rate, solving the crystal structure of a GS-like effector suggests this is not a loss, but rather a diversification of function. A key residue required for catalysis is 100% conserved in a functionally relevant position, in itself implying function. Further, all GS-like effectors tested still bind ATP, and have a functioning ATP-grasp fold comprised of a two helix “lid” which closes over the active site upon ATP-binding. Together, this suggest they have not lost catalytic activity but have diversified to a novel substrate. In support of this hypothesis, GS-like effectors vary in their substrate binding pocket compared to true GS enzymes, and therefore probably represent a novel biosynthetic pathway unique to a specific group of plant parasitic nematodes. This opens the possibility of effector-informed drug design against a target specific to these species.



## **Comprehensive *Transcriptome* Profiling of Root-knot Nematodes During Plant Infection and Characterization of Species-Specific Traits**

Nguyen C.-N, Perfus-Barbeoch L., M.Quentin, Danchin, E.G.J, Da Rocha M Magliano, M. Abad, P and Favery B.

*INRA-UNS-CNRS, UMR Institut Sophia Agrobiotech, FR-06600 Sophia Antipolis, France*

Root-knot nematodes (RKN) are obligate endoparasites that maintain a biotrophic relationship with their hosts over a period of several weeks. They infect roots as microscopic vermiform second-stage juveniles (J2) and migrate between cells to reach the plant vascular cylinder. To further develop and molt into a pear-shaped female that will release hundreds of eggs on the root surface, J2s need to successfully establish and maintain specialized feeding structures called “giant-cells” from which they withdraw water and nutrients. Our project aims to identify RKN genes specifically involved in plant parasitism with an emphasis on genes encoding new secreted effectors. Using Illumina RNA-seq technologies, we compared transcriptomes of *Meloidogyne incognita* during its life cycle and identified genes over-expressed in early parasitic stages as compared to pre-parasitic juveniles (J2s), eggs, females and males. Once the over-expression of selected genes in parasitic stages was confirmed by RT-qPCR, in situ hybridizations were carried out to localize the candidates in the nematode secretion organs. Furthermore, siRNA soaking was used to silence these genes and study their role in pathogenicity. In parallel, we are also comparing the transcriptomes of *M. incognita* with those of another RKN species that reproduces by obligatory parthenogenesis, *M. enterolobii*. This nematode represents a new threat for the agriculture worldwide because of its ability to reproduce on the majority of known RKN-resistant plants. This comparison will allow us to identify, not only the common set of effectors, but also those specific to one of the other RKN species and possibly involved in host range differences.

## **In planta identification of putative pathogenicity factors from the chickpea pathogen *Ascochyta rabiei***

Nicolas Krezdorn<sup>2</sup>, Björn Rotter<sup>2</sup>, Peter Winter<sup>2</sup>, Günter Kahl<sup>3</sup>, Sara Fondevilla<sup>1,3</sup>

*1 Institute for Sustainable Agriculture- CSIC, Avda. Menendez Pidal s/n, 14004, Córdoba, Spain*

*2 GenXPro GmbH, Altenhöferallee 3, D-60438, Frankfurt am Main, Germany*

*3 University of Frankfurt, Biocenter, Max-von Laue Str. 9, D-60438 Frankfurt am Main, Germany*

The most important foliar diseases in legumes worldwide are ascochyta blights. Health or disease is the result of a battle between plants and their pathogens. However, in the Ascochyta-legume pathosystem most studies focused on the identification of resistance genes in the host, while very little is known about the pathogenicity factors of Ascochyta spp. This study aimed at the identification of pathogenicity factors of ascochyta blight pathogens using *Ascochyta rabiei* as a model. Towards this objective we used NGS for the de novo sequencing of the *A. rabiei* transcriptome, and to identify genes differentially expressed by the fungus during infection of chickpea leaves in comparison to the fungus growing under artificial conditions. Combining RNA-Seq and MACE data we generated a comprehensive transcriptome data base comprising 22,725 assembled *A. rabiei* contigs with an average length of 1178 bp. Since pathogenicity factors are usually secreted, we predicted the *A. rabiei* secretome, yielding 550 putatively secreted proteins. Accurate transcriptome quantification by MACE identified 597 transcripts that were up-regulated during infection. An analysis of these genes identified a collection of candidate pathogenicity factors and effectors such as cell wall-degrading enzymes, toxins and genes involved in the detoxification of fungitoxic compounds produced by the plant as a defense.

## **Functional analyses of putative determinants of host specialization and pathogenicity in *Zymoseptoria tritici***

Sharon Bahena-Garrido<sup>1,2</sup>, Franziska Scheidemantel<sup>2</sup>, Petra Happel<sup>2</sup>, Ronny Kellner<sup>2</sup> and Eva H. Stukenbrock<sup>1,2</sup>

*1 Environmental Genomics, Botanical Institute, Christian-Albrechts University of Kiel, Germany and Max Planck Institute for Evolutionary Biology, Plön, Germany*

*2 Max Planck Institute for Terrestrial Microbiology, Marburg, Germany*

How plant pathogens adapt to a specific host remains one of the important questions in the field of phytopathology and ecological genetics. One way to understand the underlying molecular mechanism of host specialization and pathogenicity is through the identification and functional analysis of candidate determinants of virulence in a given specific host. We took a transcriptomic approach to identify genes specifically upregulated in a compatible host-pathogen interaction versus a non-compatible interaction of the hemibiotrophic fungus *Zymoseptoria tritici*. *Z. tritici* is specialized to infect wheat *Triticum aestivum* while it cannot establish infectious hyphae in the mesophyll of the grass species *Brachypodium distachyon*. Based on the RNAseq data at an early and critical stage of infection (4 d.p.i.) on wheat and *B. distachyon*, we identified four genes (Zt69330, Zt42222, Zt41440 and Zt107320) that were significantly differentially expressed between the two hosts suggesting a determining role in host compatibility. These putative determinant genes have putative functions related to pathogenicity. Zt69330 encodes a glycoside hydrolase; Zt42222, a multicopper oxidase; Zt41440 and Zt107320 are both Zn(II)2Cys6 transcription factors. Using a reverse genetic approach, we analyze the functional roles of the four genes in the *Z. tritici*-wheat interaction. Plant infection assays of the mutant strain  $\Delta$ Zt69330 reveal a reduced degree of necrosis formation in the mutant supporting a role of Zt69330 in host cell wall degradation and necrotrophic establishment of *Z. tritici* in wheat. The finding of virulence related genes from the RNAseq datasets from *T. aestivum* and *B. distachyon* underlines the power of comparative transcriptome profiling in the search of virulence determinants in pathogens.

## **Molecular basis and origin of Co-x, an atypical disease resistance gene to anthracnose in common Bean**

Manon Richard and Valerie Geffroy

*Institute of Plant Sciences Paris Saclay IPS2, CNRS, INRA, Université Paris-Sud, Université Evry, Université Paris Diderot, Université Paris-Saclay, Batiment 630, 91405 Orsay, France*

Plant resistance to microbial pathogens is a complex process relying on different layers of resistance. Specific resistance relies on the specific recognition of pathogen-derived effectors, called Avirulence (Avr) proteins, by plant resistance (R) proteins encoded by R genes. Strikingly, the majority of cloned R genes encodes Nucleotide Binding-Leucine Rich Repeat (NB-LRR) proteins. Anthracnose, caused by the phytopathogenic fungus *Colletotrichum lindemuthianum*, is one of the most important diseases of common bean. Various specific resistance (R) genes, named Co-, conferring race-specific resistance to different strains of *C. lindemuthianum* have been identified. The Andean cultivar JaloEEP558 was reported to carry Co-x, conferring resistance to a highly virulent strain of *C. lindemuthianum*. Access to the complete genome sequence of the Andean genotype G19833 provides the opportunity to rapidly develop locus-specific markers for map-based cloning strategy. To fine map Co-x, 181 recombinant inbred lines (RILs) derived from the cross between JaloEEP558 and BAT93 were genotyped with PCR-based markers developed using the genome sequence of G19833. In this report, we will present the molecular basis of Co-x, that is not a typical NB-LRR encoding gene.

## **Exploitation of the knowledge on oomycete effectors to drive the discovery of durable disease resistance in cultivated plants: the case of *Plasmopara halstedii*, the agent of sunflower downy mildew**

Yann Pecrix, Luis Buendia, Quentin Gascuel, Charlotte Penouilh-Suzette, Laurence Godiard

*Laboratoire des Interactions Plantes-Microorganismes (LIPM), INRA-CNRS INRA Toulouse, F-31326 Castanet-Tolosan, France*

*Plasmopara halstedii* is an obligate biotroph oomycete causing downy mildew disease on sunflower, *Helianthus annuus*, an economically important cultivated crop. Disease symptoms observed in fields, plant dwarfism, leaf bleaching, sporulation and production of infertile flowers, impair strongly seed yield. *P. halstedii* pathotypes are defined by their divergent virulence profiles in a set of sunflower differential hosts carrying different PI resistance genes, not yet cloned. Number of pathotypes increased from 1 to 16 during the last 25 years in France, concomitantly with the breakdown of PI resistance loci used in fields. Finding broad-spectrum a priori durable resistance against pathogens would open the doors to efficient, environmentally friendly and cost-effective disease control. In oomycetes, two classes of effectors are translocated into the host plant, RXLRs and CRNs, but oomycete avirulence genes described so far are RXLRs. Through high throughput genomic sequencing of 17 *P. halstedii* pathotype isolates, we selected by stringent in silico methods, 74 putative RXLR effectors. 33 show polymorphism with at least one pathotype whereas 41 are conserved in sequence among the 17 pathotypes. Analysing the pathotype effector polymorphism in regard to the content in PI resistant genes of sunflower lines should help us to identify candidates for pathogen avirulence genes. Triggering of defense reactions (Hypersensitive Response) through their transient expression in sunflower lines carrying known resistance genes will be used to validate them. Subcellular localization experiments of selected candidate effectors fused to GFP should give hints to their function in the plant cell. In addition, polymorphic effectors will be used to design molecular markers for rapid pathotype identification. The 35 conserved effectors correspond to highly expressed genes upon sunflower infection and are suspected to be essential genes for the pathogen. They are tested by agroinfiltration on various resistance sources of *H. Annuus* and some of them induce plant cell death. Co-segregation of resistance with cell death activity caused by the effector will have to be tested on segregating populations. If true, these effectors should accelerate the identification, the functional characterization and the mapping of broad-spectrum sunflower resistances potentially sustainable.

## **Analysis of evolutionary adaptation of HopQ1 effector from *Pseudomonas syringae* to given plant host species**

Fabian Giska<sup>1</sup>, Lennart Eschen-Lippold<sup>2</sup>, Marcin Piechocki<sup>1</sup>, Monika Kaluzna<sup>3</sup>, Piotr Sobiczewski<sup>3</sup>, Justin Lee<sup>2</sup>, Jacek Hennig<sup>1</sup>, Magdalena Krzymowska<sup>1</sup>

*1 Institute of Biochemistry and Biophysics PAS, Warsaw, Poland*

*2 Leibniz Institute of Plant Biochemistry, Halle (Saale), Germany*

*3 Institute of Horticulture, Skierniewice, Poland*

How plant pathogens adapt to a specific host remains one of the important questions in the field of phytopathology and ecological genetics. One way to understand the underlying molecular mechanism of host specialization and pathogenicity is through the identification and functional analysis of candidate determinants of virulence in a given specific host. We took a transcriptomic approach to identify genes specifically upregulated in a compatible host-pathogen interaction versus a non-compatible interaction of the hemibiotrophic fungus *Zymoseptoria tritici*. *Z. tritici* is specialized to infect wheat *Triticum aestivum* while it cannot establish infectious hyphae in the mesophyll of the grass species *Brachypodium distachyon*. Based on the RNAseq data at an early and critical stage of infection (4 d.p.i.) on wheat and *B. distachyon*, we identified four genes (Zt69330, Zt42222, Zt41440 and Zt107320) that were significantly differentially expressed between the two hosts suggesting a determining role in host compatibility. These putative determinant genes have putative functions related to pathogenicity. Zt69330 encodes a glycoside hydrolase; Zt42222, a multicopper oxidase; Zt41440 and Zt107320 are both Zn(II)2Cys6 transcription factors. Using a reverse genetic approach, we analyze the functional roles of the four genes in the *Z. tritici*-wheat interaction. Plant infection assays of the mutant strain  $\Delta$ Zt69330 reveal a reduced degree of necrosis formation in the mutant supporting a role of Zt69330 in host cell wall degradation and necrotrophic establishment of *Z. tritici* in wheat. The finding of virulence related genes from the RNAseq datasets from *T. aestivum* and *B. distachyon* underlines the power of comparative transcriptome profiling in the search of virulence determinants in pathogens.

## **P24: Paolo IOVIENO**

### **Identification of candidate MLO powdery mildew susceptibility genes in *Cucurbita Pepo* and functional characterization in tomato**

Paolo Iovieno, Giuseppe Andolfo, Maria Mafalda Barbella, Stefano Pavan, Luigi Frusciante, Maria Raffaella Ercolano,

*Department of Agriculture Sciences, University of Naples 'Federico II', Via Università 100, 80055 Portici (Naples), Italy*  
*Department of Soil, Plant and Food Science, University of Bari "Aldo Moro", Via Amendola 165/A, 70126 Bari, Italy*

The powdery mildew disease affects thousands of plant species and represents the major fungal threat for crops such as zucchini (*Cucurbita pepo* L.). Several studies revealed that specific members of the Mildew Locus O (MLO) gene family act as powdery mildew susceptibility factors. Noteworthy, TALEN and CRISPR/Cas9 technology have been recently used to knock out these genes introducing a particular form of resistance referred as mlo resistance. We exploited recently available genomic information reporting the identification of 18 MLO homologs in *C. pepo* genomes. Phylogenetic inference and the detection of microsyntenic regions allowed us to reconstruct several relations of orthology between zucchini Mlo genes. Evolutionary and sequence relatedness with functionally characterized MLO homologs resulted in the identification of candidate powdery mildew susceptibility factors. Finally, we carried out a comprehensive codon-based evolutionary analysis of homologs putatively implicated in susceptibility indicating a general high level of purifying selection and regions under diversifying selection. Furthermore, we confirmed the role of zucchini MLO homology in powdery mildew pathogenesis transforming a mlo tomato mutant to obtain overexpressing transgenic plants. In parallel, we used CRISPR/Cas9 technologies in *Cucurbita Pepo* to introduce targeted mutations in two CpMLOs developing CRISPR/Cas9 constructs. Results of this study reported here could be conveniently used by breeding research, aiming to select powdery mildew resistant cultivars in zucchini.

## **P25: Marjin KNIP**

### **Studying the link between DNA-damage and NLR-mediated immune responses**

Marijn Knip<sup>1</sup>, Martin Cann<sup>2</sup>, Frank Takken<sup>1</sup>

*1 Molecular Plant Pathology, SILS, University of Amsterdam, The Netherlands*

*2 School of Biological and Biomedical Sciences, Durham University, Durham, United Kingdom*

Nucleotide-binding Leucine-rich Repeat proteins (NLRs) are a highly conserved, major class of immune receptors. We're developing a system that allows studying the newly discovered ability of NLRs to bind and nick DNA in planta in relation to the onset of immune responses. NLR-type immune receptors are present in both the nucleus and the cytosol and require both localizations to be fully functional. Very little is known about the molecules targeted by NLR's in immunity. In collaboration with the Cann laboratory, our group has recently found that NLRs can bind and nick DNA in vitro<sup>1</sup>. It was also found that DNA binding occurs in vivo, and relies on the activation of the NLR receptor following recognition of its genuine effector<sup>1</sup>. These findings could provide an explanation why NLR-receptors require a nuclear localization to function: Activated NLRs might trigger immune responses by directly binding and/or nicking DNA. To study the nuclear function of NLR proteins, we're developing a system that allows synchronized induction of NLR-triggered immune responses, by using *N. benthamiana* plants that constitutively express the potato NLR-protein Rx1 in combination with controlled expression of the PVX-coat protein. This system allows us to study the occurrence, localization and timing of NLR-induced DNA-damage using TUNEL and COMET assays and to monitor the onset of subsequent specific immune responses by, among other methods, qPCR.

## **Elucidation of mechanisms underlying virulence function of *Pseudomonas syringae* HopQ1 effector in plant cells**

Jakub Kwiatkowski , Fabian Giska, Marcin Piechocki, Rafal Hoser, Jacek Hennig, Magdalena Krzymowska

*Institute of Biochemistry and Biophysics PAS, Warsaw, Poland*

HopQ1 (for Hrp outer protein Q), a TTSS effector secreted by *Pseudomonas syringae* promotes virulence of bacteria in many plant species. Mechanisms underlying HopQ1 function remain largely elusive, however, we have recently determined a few factors, that seem to regulate HopQ1 activity in plant cells. First, after delivery into plant cells HopQ1 is phosphorylated and binds host 14-3-3 proteins, which stabilize the effector, affect its subcellular localization and possibly facilitate its interaction with other host proteins. Furthermore, redox conditions and calcium ion level appear to play an important role in the control of HopQ1 oligomerization and subcellular localization. Size exclusion chromatography coupled to MALS (Multi-Angle Light Scattering) revealed that HopQ1 forms in vitro monomers, dimers and trimers. However, treatment with a reducing agent or mutations in either one of both HopQ1 cysteines abolishes oligomerization, indicating that HopQ1 oligomeric state depends on disulfide bridge formations. On the contrary, under calcium depleted conditions, HopQ1 monomers are reversibly converted to dimers. Similarly mutations within the predicted calcium binding motif (HopQ1-D107A\_D108A) produced HopQ1 dimers in vitro and in planta. Our data indicate that dimer assembly promotes nuclear localization of HopQ1. Preliminary experiments show that binding of calcium ions in vitro, results in an increased melting temperature of HopQ1. Collectively, these results suggest that calcium may affect localization and stability of the effector. We hypothesize that calcium ions might be also involved in regulation of HopQ1 interaction with specific targets in plant cells. The studies that address this problem are now under way.



## **Wheat Effector Assisted Breeding for Resistance to Fungal Pathogens (WEAB)**

Thierry Langin<sup>2</sup>, Thomas Kroj<sup>3</sup>, James Cockram<sup>4</sup>, Richard Oliver<sup>5</sup>, Gert Kema<sup>6</sup>, Romain Valade, Sébastien Praud<sup>8</sup>, Valérie Laurent<sup>9</sup>, Laure Duchalais<sup>10</sup>, Marc-Henri Lebrun<sup>1</sup>

*1 INRA BIOGER, Thiverval-Grigno, France*

*2 INRA GDEC, Clermont-Ferrand, France*

*3 INRA BGPI, Montpellier, France*

*4 NIAB, United Kingdom*

*5 CCDM, Australia*

*6 PRI, The Netherlands*

*7 BIOGEMMA, Clermont-Ferrand, France*

*8 Florimond-Desprez, Cappelle en Pévèle, France*

*9 RAGT, Louville la Chenard, France*

*10 Volker LEIN, CETAC, Estrées-Saint-Denis, France*

The discovery that fungal effector proteins are important for infection represents a novel opportunity for controlling plant diseases. Use of fungal effectors for resistance breeding is a game-changing technology creating opportunities and innovative methods to identify novel resistances to fungal diseases in plants. These methods are amenable to high throughput phenotyping. The recent availability of high-density genetic marker coverage of the wheat genome allows the mapping of novel resistances identified through such high throughput phenotyping. We are using necrotrophic protein effectors from *Parastagonospora nodorum* (Pn) and toxic proteins from *Fusarium graminearum* (Fg) and *Zymoseptoria tritici* (Zt) to detect resistance genes/QTLs in wheat. Complementary strategies will be used to detect a large array of resistance mechanisms to fungal effectors. Recombinant necrotrophic protein effectors and toxic proteins are produced in yeast and the purified proteins are delivered into wheat leaves by syringe infiltration. Symptom development is scored few days after infiltration. Screening of 220 elite French wheat cultivars with Pn ToxA, 1 and 3 has highlighted a large number of cultivars insensitive to the 3 necrotrophic effectors, and only a few cultivars that were sensitive to all three effectors, suggesting that previous breeding for field resistance to Pn (1960-1980) led to the accumulation of insensitivity alleles. To validate this hypothesis, we are currently pathotyping these wheat cultivars with a French Pn isolate producing Tox1 and 3. Mapping of loci controlling insensitivity to Pn necrotrophic effectors and resistance to Pn isolate will be performed using genome-wide association analyses. This project will facilitate plant breeding efforts to select for resistance to important fungal pathogens by providing a 'toolkit' of bio-molecular markers.

## **Chitinase of *Drosera rotundifolia* in transgenic tobacco protein extracts suppressed the growth of *Fusarium poae* in hyphal extension assay**

Dominika Durechova, Martin Jopcik, Miroslav Rajnec, Jana Libantova

*Institute of Plant Genetics and Biotechnology, Slovak Academy of Sciences, Akademicka 2, P. O. Box 39A, 950 07 Nitra, Slovak Republic*

Here, we focused on the investigation of the carnivorous plant chitinase gene potential for protection of the crops against to fungal pathogens. For this, the gene of class I extracellular chitinase upregulated during digestive processes of sundew was introduced into the *Nicotiana tabacum* L. via *Agrobacterium*-mediated transformation. The introduced gene was constitutively expressed in transgenics and ninety micrograms of crude protein extracts were tested for capability to inhibit the growth of *Fusarium poae*, *F. tricinctum* and *F. oxysporum*, respectively, in hyphal extension assay. The results showed that the extracts of most transgenics, unlike of non-transgenic control, exerted inhibition zone when the proteins were in contact with fungi. From tested phytopathogens, *F. poae* showed the strongest sensitivity to the presence of protein extracts containing sundew chitinase. When the individual transgenics were compared, the strongest antifungal potential showed the two-copy number line DD3/3 and single-copy line DD3/9. Lines DD3/1, DD3/2 and DD3/7 exerted also obvious antifungal activity to the *F. poae*, however, in hyphal extension-inhibition assay with *F. oxysporum* and *F. tricinctum* the inhibition zones did not reach the level of lines DD3/3 and DD3/9.

## **Comparative Secretome Analysis of *Ralstonia solanacearum* Type 3 Secretion-Associated Mutants Reveals a Fine Control of Effector Delivery, Essential for Bacterial Pathogenicity**

Fabien Lonjon<sup>1,2</sup>, Marie Turner<sup>1,2</sup>, Céline Henry<sup>3</sup>, David Rengel<sup>1,2</sup>, David Lohou<sup>1,2</sup>, Quitterie van de Kerkhove<sup>1,2</sup>, Anne-Claire Cazalé<sup>1,2</sup>, Nemo Peeters<sup>1,2</sup>, Stéphane Genin<sup>1,2</sup>, Fabienne Vailleau<sup>1,2,4</sup>

1 INRA, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR441, 31326 Castanet-Tolosan, France

2 CNRS, Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR2594, 31326 Castanet-Tolosan, France

3 UMR 119 MICALIS, PAPPISO, Domaine de Vilvert, 78352 Jouy en Josas, France

4 Université de Toulouse; INP; ENSAT; 18 chemin de Borde Rouge, 31326 Castanet Tolosan, France

*Ralstonia solanacearum*, the causal agent of bacterial wilt, exerts its pathogenicity through more than a hundred secreted proteins, many of them depending directly on the functionality of a type III secretion system. Regulation of type III effectors expression at the transcriptional level is well understood, however little is known post-translational regulations of type III secretion system substrates. We identified four proteins potentially involved in the control of type III effectors secretion: the chaperones HpaB and HpaD, the Type III secretion substrate specificity switch (T3S4) protein HpaP and the LRR protein HpaG. In order to investigate how type III secretion is regulated at the post-translational level and to identify the role of these proteins on type III effectors secretion, we analyzed the secretome of the wild type strain as well as the four hpa mutants using mass spectrometry experiments. We described the most exhaustive secretome analysis of a plant pathogenic bacterium using a MS-based shotgun approach. This analysis allowed the identification of RipBJ, a new *R. solanacearum* type III effector. Concerning the hpa mutants, this analysis revealed different type III secretion patterns, highlighting specific subsets of effectors differentially secreted in some of the mutant strains. The pathogenicity of these *R. solanacearum* mutants was evaluated on several plants, and interestingly, different host specificities could be identified. Advantages of such a global approach to highlight sets of T3Es potentially required for *R. solanacearum* pathogenicity on hosts belonging to diverse botanical families (solanaceous, legumes...) will be discussed.

## **MicroRNA-mediated regulation of R genes involved in the plant response against *Pseudomonas syringae***

Diego López Márquez, Rodríguez-Negrete EA, Zumaquero A, Bejarano ER, Beuzón CR

*Instituto de Hortofruticultura Subtropical y Mediterránea "La Mayora", Universidad de Málaga-Consejo superior de investigaciones Científicas (IHSM-UMA-CSIC). Dpto. Biología Celular, Genética y Fisiología, Campus de Teatinos, 29071, Málaga, Spain*

There are two main types of noncoding small RNA molecules in plants, classified as microRNAs (miRNAs) and small interfering RNAs (siRNAs), which differ in their biogenesis and mode of action, but share similar sizes (20-24 nt). The precursors of these small RNAs, are processed by Dicer-Like RNase III (dcl) proteins present in *Arabidopsis thaliana*, and can act as negative regulators of gene expression, being involved in a vast array of plant processes, including plant development, genomic integrity or response to stress. Regulation carried out through these small RNAs can occurs at transcriptional level (TGS) or at post-transcriptional level (PTGS). In recent years, the role of gene silencing in the regulation of genes related to the plant defence response against bacterial pathogens is becoming clearer. We have carried out comparisons between the expression profiles of different mutants affected in gene silencing, and plants challenged with *Pseudomonas syringae* pathovar tomato DC3000. These comparisons have led us to identify a set of uncharacterized R genes, belonging to the TIR-NBS-LRR gene family, which are expressed differentially in both conditions. Using bioinformatics, we have found a miRNA\* responsible for the regulation of the expression levels of these R genes, through the generation of siRNAs. In addition we identify one of these genes as a negative regulator of defence response against *Pseudomonas syringae*.

### **P31: Takaki MAEKAWA**

## **Convergent targeting of a host-signalling pathway by unrelated pathogen effectors and their surveillance by allelic immune receptors**

Takaki Maekawa, Xunli Lu, Barbara Kracher, Paul Schulze-Lefert

*Max Planck Institute for Plant Breeding Research, Department of Plant-Microbe Interactions, 50829 Köln, Germany*

A co-evolutionary arms race between the host barley and the pathogenic powdery mildew fungus *Blumeria graminis* f. sp. *hordei* (Bgh) has driven the functional diversification of the Mildew locus A (MLA) gene in the host population. As a consequence, allelic NLR-type MLA receptor variants each recognize a cognate isolate-specific Bgh effector encoded by AVRA genes. Diversification of allelic NLR variants is thought to be the result of a co-evolutionary arms race mediated by iterative cycles of receptor and pathogen effector adaptations, which predicts direct receptor-effector associations. Allelic MLAs were thus expected to directly bind allelic AVRA proteins. We recently isolated five AVRA loci using genome-wide association mapping of transcripts from different Bgh isolates. Unexpectedly the AVRA proteins are sequence-unrelated and a yeast-two hybrid experiment failed to detect direct receptor-effector associations. Thus we postulate an indirect AVRA recognition mechanism confers the MLA-mediated resistance. We also noted that diverse molecular processes at the AVRA loci (e.g. amino-acid substitution, transposable element insertion, loss of transcript) impair their avirulent functions in the Bgh population. Remarkably, a MLA ortholog in wheat, a sister species of barley, confers resistance to the wheat stem rust pathogen *Puccinia graminis* tritici (Pgt) Ug99. Thus, Bgh and Pgt effectors likely converge on the same host-signalling pathway needed for pathogenesis in plants lacking matching MLAs and the host-signalling pathway is monitored by MLA receptors.

---

### **P32: Johana C MISAS VILLAMIL**

## **A fungal effector reveals new mechanisms in the inhibition of Cysteine proteases**

Johana C Misas Villamil<sup>1</sup>, André N. Mueller<sup>1</sup>, Christian Pichlo<sup>2</sup>, Magdalena Schacherl<sup>2</sup>, Gunther Doehlemann<sup>1</sup>

*1 Botanical Institute and Cluster of Excellence on Plant Sciences, University of Cologne, 50674 Cologne, Germany*

*2 Institute of Biochemistry, University of Cologne, Otto-Fischer-Strasse 12-14, 50674 Cologne, Germany*

*Ustilago maydis* is a fungal biotroph responsible of the corn smut in maize. To manipulate its host, *U. maydis* secretes a set of effectors into the apoplast downregulating plant immune responses. One of these effectors is Pit2, a secreted cysteine protease inhibitor. Pit2 contains a new 14 amino acid protease inhibitor domain (PID14) that is conserved in related smut fungi and also appears in various root endophyte bacteria. The biochemical characterization of synthetic PID14 peptides based on natural mutations as well as their inhibitory profile against papain, revealed the most important amino acids required for the interaction with cysteine proteases. How cysteine proteases modulate immunity in maize and how their inhibition affects the interaction with different microorganisms are main focus of our research. The attendance to the 3rd COST conference will bring me the opportunity to share our last results in understanding the role of apoplastic cysteine proteases in immunity and to interact with specialists in the field establishing possible new collaborations.

## **Study of the genomic diversity of *Verticillium dahliae* found in naturally infested sunflower fields**

Hélène Missonnier<sup>1,2</sup>, Luigi Faino<sup>3</sup>, Virginie Mirleau-Thebaud<sup>2</sup>, Jean Daydé<sup>1</sup>, Alban Jacques<sup>1</sup> and Bart Thomma<sup>3</sup>

*1 Equipe Agrophysiologie et Agromolécules, Département des Sciences Agronomiques et Agroalimentaires, Institut National Polytechnique de Toulouse –Ecole d'Ingénieurs de Purpan, Université de Toulouse, Toulouse, France*

*2 Syngenta France S.A.S., 12 chemin de l'Hobit 31790 Saint-Sauveur, France*

*3 Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands*

Plant disease management approaches are mainly resistance genes and agrochemicals that are used repeatedly until their efficacy is overcome by the targeted pathogen. Despite no sexual cycle observed, comparative genomics show extensive chromosomal rearrangements and lineage-specific genomic regions that are increasing *V. dahliae* evolutionary potential. One aspect of *V. dahliae* that remains poorly studied, and thus also unknown, is to what extent the genetic make-up of the pathogen presents in field is uniform, or whether various pathogen strains are present simultaneously, each with their own patchy distribution. Yet, the complex relationship between spatial pattern, crop history and genetics and the evolutionary dynamic of *Verticillium* populations are not well understood. Results from 3-years experiments on native sunflower-*V. dahliae* pathosystem showed a disease expression gradient in the fields investigated with no correlation with primary inoculum distribution. French and Argentinian field isolates of *V. dahliae* were sampled according to a design developed from the observations. First experiment to measure diversity and identify D/ND pathotypes and Race 1/2 pathotypes was performed. From this study, a panel of isolates was fully sequenced to allow a reductionist approach that implies sequencing of pooled samples by targeting genomic regions that are known to be variable among different strains. Then, we expect to be able to determine potential aggressiveness differences, and link these to host-specific effectors.

## **The molecular bases of recognition of the *M. Oryzae* effector protein AVR-Pia by the rice immune receptor RGA5**

Diana Ortiz<sup>1</sup>, Karine de Guillen<sup>2</sup>, Véronique Chalvon<sup>1</sup>, Andre Padilla<sup>2</sup>, Thomas Kroj<sup>1</sup>

*1 BGPI - INRA- CIRAD - Montpellier SupAgro, UMR 0385 34000 Montpellier, France*

*2 CBS Centre de Biochimie Structurale, INSERM U1054, CNRS UMR5048, University of Montpellier, Montpellier, France*

Plant immune receptors of the NLR class are multi domain proteins characterized by an N-terminal TIR or coiled-coil domain, a central nucleotide-binding domain and an N-terminal leucine-rich domain. NLRs act by recognizing pathogen effector proteins in the plant cytosol either by direct binding or in an indirect manner. Despite the cloning of the first plant NLRs more than 20 years ago, the molecular bases of effector recognition remain badly defined. Here we used a structure-aided approach to elucidate the molecular recognition mechanisms of the AVR-Pia effector protein from the blast fungus *Magnaphorte oryzae* by its cognate NLR receptor RGA5 from rice. AVR-Pia binds directly to an uncommon C-terminal domain of RGA5 that is homologous to the copper chaperone ATX1 (Related to ATX1 domain or RATX1 domain). By using recombinant AVR-Pia and ATX1 proteins, the affinity of binding was determinate by in vitro binding experiments and the AVR-Pia binding surface was delimited by NMR titration experiments. Yeast two hybrid and in planta protein-protein interaction studies with AVR-Pia mutant proteins confirmed this interaction surface and identified amino acids of AVR-Pia that are crucial for RATX1 binding. The importance of these amino acids for effector recognition during rice infection was confirmed with transgenic *M. oryzae* isolates expressing AVR-Pia mutant variants. This study sheds new light on NLR function and opens the way to a molecular understanding of effector recognition in cereals.

## **Using comparative transcriptomics to identify new virulence factors in the wheat pathogen *Zymoseptoria tritici***

Javier Palma Guerrero, Daniel Croll, Stefano Torriani, Marcello Zala, Bruce McDonald

*Plant Pathology Group, Institute of Integrative Biology, ETH Zurich, Switzerland*

*Zymoseptoria tritici* (previously known as *Mycosphaerella graminicola*) is an ascomycete fungus that causes Septoria Tritici Blotch (STB), an important foliar disease on wheat. An epidemic of STB can reduce yields by 30-50%. *Z. tritici* is the most important wheat pathogen in Europe and it is among the three most important pathogens of wheat in the USA. *Z. tritici* is a highly polymorphic species with significant intraspecific variation in virulence profiles. It also has an unusual life history. *Z. tritici* is a hemibiotroph and, unlike most plant pathogens, it infects plants through stomata rather than by direct penetration and also exhibits a long incubation period of up to 2-3 weeks following infection. We generated a deep transcriptome sequencing dataset spanning the entire time-course of infection of four *Z. tritici* strains isolated from a Swiss wheat field. These four strains show different virulence levels among them. By comparing the transcriptome of the four isolates we found that major components of the fungal infection transcriptome were conserved between the four strains. However, individual small secreted proteins and secreted hydrolytic enzymes showed strongly differentiated transcriptional profiles between strains. Our analyses showed that successful STB infections involve complex transcriptome remodelling to upregulate distinct gene functions. Heterogeneity in transcriptomes among isolates may be responsible for some of the considerable variation in virulence and host specialization found within the species.



## **Functional Assignment to Positively Selected Sites in the Core Type III Effector RipG7 from *Ralstonia solanacearum***

Keke Wang<sup>1,2</sup>, Philippe Remigi<sup>1,2</sup>, Maria Anisimova<sup>3</sup>, Fabien Lonjon<sup>1,2</sup>, Ilona Kars<sup>1,2</sup>, Andrey Kajava<sup>4</sup>, Chien-Hui Li<sup>5</sup>, Chiu-Ping Cheng<sup>5</sup>, Fabienne Vailleau<sup>1,2,6</sup>, Stéphane Genin<sup>1,2</sup>, Nemo Peeters<sup>1,2</sup>

1 INRA, Laboratoire des Interactions Plantes Micro-organismes (LIPM), UMR441, Castanet-Tolosan, France

2 CNRS, Laboratoire des Interactions Plantes Micro-organismes (LIPM), UMR2594, Castanet-Tolosan, France

3 Institute of Applied Simulations, School of Life Sciences and Facility Management, Zürich University of Applied Sciences, Wädenswil, Switzerland

4 Centre de Recherche de Biochimie Macromoléculaire, CNRS UMR5237, 1919 route de Mende, Montpellier, France

5 Institute of Plant Biology, National Taiwan University, Taipei, Taiwan

6 Université de Toulouse; INP; ENSAT; 18 chemin de Borde Rouge, 31326 Castanet-Tolosan, France

The soil-borne pathogen *Ralstonia solanacearum* causes bacterial wilt in a broad range of plants. The main virulence determinants of *R. solanacearum* are the Type III Secretion System (T3SS) and its associated Type III Effectors (T3Es), translocated into the host cells. Among the conserved T3Es among *R. solanacearum* strains, The Fbox protein RipG7 is required for *R. solanacearum* pathogenesis on *Medicago truncatula*. In this work we describe the existing natural ripG7 variability existing in the *R. solanacearum* species complex. We show that eight representative ripG7 orthologs have different contributions to pathogenicity on *M. truncatula*: only ripG7 from Asian or African strains can complement the absence of ripG7 in GMI1000 (Asian reference strain). Nonetheless, RipG7 proteins from American and Indonesian strains can still interact with *M. truncatula* SKP1-like/MSKa protein, essential for the function of RipG7 in virulence. This indicates that the absence of complementation is most likely due to variability in the leucine-rich repeat domain (LRR) of RipG7. We identified eleven sites under positive selection in the LRR domains of RipG7. By studying the functional impact of those 11 sites, we show the contribution of 5 positively selected sites for the function of RipG7CMR15 in *Medicago truncatula* colonization. This work reveals the genetic and functional variation of the essential core T3E RipG7 from *R. solanacearum*. This analysis is the first of its kind on an essential disease-controlling type III effector and sheds light on the co-evolutionary arms race between the bacterium and its hosts.

## **Insight into the diversity of plant resistance mechanisms to viruses through the Rice-Rice yellow mottle virus pathosystem**

Hélène Pidon<sup>1</sup>, Agnès Pinel<sup>2</sup>, Eugénie Hébrard<sup>2</sup>, Sophie Chéron<sup>1</sup>, Alain Ghesquière<sup>1</sup> and Laurence Albar<sup>1</sup>

*1 IRD, UMR DIADE, F-34394 Montpellier 5, France 2IRD, UMR IPME, F-34394 Montpellier 5, France*

*2 IRD, UMR IPME, F-34394 Montpellier 5, France 2IRD, UMR IPME, F-34394 Montpellier 5, France*

The Rice yellow mottle virus (RYMV, Sobemovirus) represents one of the major threats for rice cultivation in Africa and Madagascar. We identified distinct high resistance pathways, associated at least with three different genes that pinpoint the diversity of mechanisms controlling plants resistance to viruses. The first gene, RYMV1, involves a mechanism specific of plant/virus interactions. It codes a translation initiation factor and implies an impaired interaction between this factor and the viral VPg protein (Albar et al., 2006). The two others genes are more probably related to the control of generic defense responses developed against diverse pathogens. The resistance allele on RYMV2 is actually a null allele of a putative negative regulator of active defense mechanisms (CPR5), probably resulting in a constitutive resistance reaction (Orjuela et al., 2013). We also recently mapped the first dominant resistance gene against RYMV, RYMV3, in a 20 kb interval containing a CC-NBS-LRR gene, that is currently under characterization. Besides, we recently identified three distinct viral determinants, that are involved in the resistance-breakdown of the three resistance genes. Specific mutations in the viral VPg protein can restore its interaction with the product of RYMV1 in resistant plants and therefore the virulence (Hébrard et al., 2010). The viral protease and the coat protein are candidates as determinants of RYMV2 and RYMV3 resistance-breakdown, respectively. Moreover, the patterns of resistance-breakdown of these different genes by isolates representative of the viral diversity show unexpected similarities that may reveal a local adaptation of the virus to its host.

## **Determining tomato apoplast responses to *Ralstonia solanacearum* by Activity-based protein profiling**

Marc Planas<sup>1</sup>, Judith Paulus<sup>3</sup>, Renier A. L. van der Hoorn<sup>3</sup>, Marc Valls<sup>1,2</sup> and Núria Sánchez Coll<sup>1</sup>

*1 Centre for Research in Agricultural Genomics (CSIC-IRTA-UAB-UB), Bellaterra, Catalonia, Spain*

*2 Genetics Department, Universitat de Barcelona, Barcelona, Catalonia, Spain*

*3 Plant Chemetics Laboratory, Department of Plant Sciences, University of Oxford, OX1 3RB Oxford, United Kingdom*

*Ralstonia solanacearum* is a soil-borne pathogen that causes the widespread disease known as bacterial wilt. This devastating disease threatens tropical and subtropical crops causing huge economical losses (Peeters et al., 2013). Once a plant is infected, there is no way of stopping the spread of the disease and, to date, there are no effective treatments that help eradicate this pathogen in the fields. The most effective way to fight this pathogen seems to be the use of resistant cultivars, but little is known about the mechanisms of plant resistance to *R. solanacearum*. To approach this issue, we analysed the response generated in the apoplast of a susceptible (Marmande) and resistant (Hawaii7996) varieties of tomato (*Solanum lycopersicum*) when challenged with *R. solanacearum*. Vacuum-infiltration of tomato leaves with this bacteria resulted in comparable bacterial growth in both susceptible and resistant varieties, but the last ones showed less disease symptoms by 7 days post-inoculation. Making use of the Activity-based protein profiling (ABPP) technology (Koodziejek & van der Hoorn, 2010[MP1] [NS2] ), we characterized apoplastic protein activities that might be responsible for such behaviour. ABPP assays revealed an induction of papain-like cysteine protease, serine hydrolase and glycosidase activities in the apoplast of infected plants. Further characterization of candidate enzymes would provide crucial information regarding *R.solanacearum*-plant interaction.

## **Identification of epigenetic marks in the plant parasitic root-knot nematode *Meloidogyne incognita***

Pratx Loris<sup>1,2,3</sup>, Perfus-Barbeoch Laetitia<sup>1,2,3</sup>, Castagnone-Sereno Philippe<sup>1,2,3</sup>, Cosseau Céline<sup>4,5</sup>, Grunau Cristoph<sup>4,5</sup>, Abad Pierre<sup>1,2,3</sup>

1 INRA, UMR 1355 ISA, Institut Sophia Agrobiotech, Sophia-Antipolis, France.

2 CNRS, UMR 7254 ISA, Institut Sophia Agrobiotech, Sophia-Antipolis, France.

3 Université de Nice Sophia-Antipolis, UMR ISA, Institut Sophia Agrobiotech, Sophia-Antipolis, France.

4 Université de Perpignan Via Domitia, Ecologie et Evolution des Interactions (2EI), Perpignan, France.

5 CNRS, UMR5244, Ecologie et Evolution des Interactions (2EI), Perpignan, France.

Root-knot nematodes, such as *Meloidogyne incognita*, are obligatory plant parasites that constitute major agricultural pests worldwide. Our knowledge about *M. incognita*'s genetics regulation has significantly increased since genome sequencing, transcriptomic analysis and gene annotations are now available (1). However, despite this knowledge, the "classical" genetics fails to understand some phenomena occurring in our model. *M. incognita* reproduces in an asexual way by parthenogenesis without meiosis. Genetically identical individuals develop from females and form clonal populations. Although these clones share the same genetic heritage, modifications of their phenotype can be observed when they are exposed to unfavorable environments. For instance, the virulence (i.e. capacity to parasite a resistant crop) is heritable but transmitted in a non-Mendelian way (not acquired by 100% of the "clonal daughters") and could not be associated to a modification in DNA sequence (2). Epigenetic modifications can drive phenotypes by other mechanisms than genetics. These modifications are heritable, but metastable, which could change phenotypes by modifying genomic expression. We propose to test role of epigenome in the generation of phenotypic variability and consequently for microevolution towards infection success. We detailed DNA methylation and nucleosome structure, carriers of epigenetic information. We also developed a ChIP-seq assay protocol to compare post-transcriptional histone modifications between virulent and avirulent parasites; and between different developmental stages. Our preliminary data indicated that the genome of *M. incognita* is not methylated and confirmed the existence of histone modifications which represents important markers involved in gene activation or repression by modifying chromatin state. This study opens the way for analyzing the role of epigenetic mechanisms at a whole genome scale and identifying new biological processes involved in the generation of phenotypic variation in asexual organisms.

## **Carbon regulation of environmental pH by secreted small effecting molecules that modulate pathogenicity in phytopathogenic fungi**

Fangcheng Bi<sup>1,2</sup>, Shiri Barad<sup>1,3</sup>, Dana Ment<sup>1</sup>, Neta Luria<sup>1</sup>, Virginia Casado<sup>4</sup>, Nofar Glam<sup>1,3</sup>, Amit Dubey<sup>1</sup>, Jose Diaz Mínguez<sup>4</sup>, Eduardo Espeso<sup>5</sup>, Robert Fluhr<sup>6</sup>, Dov Prusky<sup>1</sup>

*1 Department of Postharvest Science of Fresh Produce, Agricultural Research Organization, the Volcani Center, Bet Dagan 50250, Israel*

*2 Institute of Fruit Tree Research, Guangdong Academy of Agricultural Sciences, Guangzhou 510640, and Key Laboratory of South Subtropical Fruit Biology and Genetic Resource Utilization, Ministry of Agriculture, Guangzhou 510640, China*

*3 Department of Plant Pathology and Microbiology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel*

*4 Department of Microbiology and Genetics, CIALE, Universidad de Salamanca, Salamanca, Spain*

*5 Department of Molecular and Cellular Biology, Centro de Investigaciones Biológicas (C.I.B.), Madrid, Spain*

*6 Department of Plant and Environmental Sciences, Weizmann Institute of Science, Rehovot, Israel*

Fruit pathogens can either acidify or alkalize the pH of the colonized host environment. This capability has been used to divide fungal pathogens into acidifying and alkalizing classes. In the present study, we show that diverse classes of fungal pathogens, including; *Colletotrichum gloeosporioides*, *Penicillium expansum*, *Aspergillus nidulans*, *Sclerotinia sclerotiorum*, and *Fusarium oxysporum*—secrete small pH-effector molecules. These molecules modify the environmental pH that dictates acidic or alkaline colonization life patterns and select for the activity of PACC-dependent genes. We show that in multiple cross-class organisms, acidification is induced under carbon excess, e.g. 175 mM sucrose. In contrast, alkalization occurs under conditions of carbon deprivation, e.g., less than 15 mM sucrose. The carbon source is metabolized by glucose oxidase (*gox2*) to gluconic acid, contributing to medium acidification. While catalyzed deamination of non-preferred carbon sources, such as the amino acid glutamate, by glutamate dehydrogenase 2 (*gdh2*) results in the secretion of ammonia. Functional analyses of *gdh2* mutants show reduced alkalization and pathogenicity during growth under carbon deprivation, but not in high-carbon media or on fruit rich in sugar, whereas analysis of *gox2* mutants show reduced acidification and pathogenicity in carbon-excess conditions. The induction pattern of *gdh2* was negatively correlated with expression of the zinc finger global carbon regulator repressor *creA*. The present results indicate that differential pH modulation by fungal pathogens is a universal host-dependent mechanism that modulates environmental pH as a tool to enhance host plant colonization.

#### **P41: Liliya PYLYPENKO**

### **Development and validation of the marker for the npr1-like *Fusarium* head blight resistance gene**

A.V. Karelova<sup>2</sup>, L.A. Pylypenko<sup>1</sup>, N.A. Kozuba<sup>2</sup>, I.A. Sozinov<sup>1</sup>, A.A. Sozinova<sup>2</sup>, Ya.B. Blume<sup>2</sup>

*1 Institute of Plant Protection, National Academy of Agrarian Sciences of Ukraine, Ukraine*

*2 Institute of Food Biotechnology and Genomics, National Academy of Sciences of Ukraine, Ukraine*

272 cultivars of common wheat (*Triticum aestivum* L.) of Ukrainian breeding were studied using the intron-localized molecular marker INDEL1 of the NPR1-like TDF\_076\_2D gene associated with moderate type II resistance to *Fusarium* head blight. It was found out that 61.7% of winter wheat cultivars and 83.3% – of spring wheat cultivars carried the resistant allele. The allelic states of the marker are not specific for different resistance associated alleles of the gene and it cannot distinguish polymorphic cultivars from the susceptible ones. Using the sequences of the alleles of the TDF\_076\_2D and TDF\_076\_2A genes (Diethelm et al., 2014) the primers complementary to the different allelic states of the TDF\_076\_2D gene were developed. As the result of the multiplex PCR 914 bp fragments were obtained in case of the resistant allele peculiar for the genotype SVP72017, 323 bp fragments – in case of the susceptible allele and no fragments – in case of resistant allele peculiar for the wheat cultivar 'Capo'. The marker was validated by analyzing 60 cultivars and the results did not contradict the ones obtained with INDEL1.

---

#### **P42: Dina RAATS**

### **Rapid isolation of new stripe rust resistance variants in cultivated wheat**

Dina Raats<sup>1</sup>, Francesca Stefanato<sup>2</sup> and Ksenia Krasileva<sup>1,2</sup>

*1 The Genome Analysis Centre, Colney Lane, Norwich, NR4 7UH, United Kingdom*

*2 The Sainsbury Laboratory, Colney Lane, Norwich, NR4 7UH, United Kingdom*

Yellow rust caused by the fungus *Puccinia striiformis* f. sp. *tritici* (Pst) is one of the most destructive diseases of wheat, resulting in yield losses of 10–70 % in susceptible varieties and total losses of harvestable grain during severe epidemics<sup>1</sup>. Recently emerged new Pst races that have expanded virulence profiles and are adapted to warmer temperatures compared to previous races are threatening over 30% of global wheat yield<sup>2</sup>. There is an urgent need to identify new sources of resistance against highly virulent races of stripe rust while lessens our dependence on fungicides. Here we propose a novel forward genetics approach to rapidly identify the genetic variants underlying the stripe rust resistance in Ethyl methanesulfonate mutagenized population of elite wheat cultivar Kronos<sup>3</sup>. Eleven mutant lines with confirmed stripe rust resistance derived from that population were backcrossed to the parental wild type. F2 populations are available for gene isolation. We will perform exome sequencing of F2 phenotypic bulks using a custom (86 Mb) wheat exome capture design developed by Krasileva et al (in preparation). Based on the frequencies analysis of SNP markers mapped to the best-ordered wheat reference genome data the chromosomal regions contain the mutations underlying the gain of resistance phenotypes will be identified. In the case the causative mutation will be missing from our initial analyses, we will further investigate the region containing the gene of interest. The gene isolation approach developed here will be applicable to any trait of interest.

## **Elucidating the mechanistic basis of *Albugo candida* mediated Immune-suppression by CCG effectors**

Amey Redkar<sup>1</sup>, Volkan Cevik<sup>1</sup>, Kate Bailey<sup>1</sup>, Oliver Furzer<sup>1</sup> and Jonathan D.G. Jones<sup>1</sup>

*1 The Sainsbury Laboratory, John Innes Centre, Norwich Research Park, Colney Lane, Norwich NR4 7UH, United Kingdom*

The oomycete pathogen *Albugo candida* infects a large number of Brassicaceae species causing white blister rust disease. Some *A. candida* races can also infect various *Arabidopsis* accessions, thus facilitating the characterization of effectors and resistance genes that are involved in this obligate biotrophic patho-system. The interactions between *A. thaliana*, *Brassica* sp. and oomycete *Albugo candida* provide an excellent model to investigate suppression of immunity. Host immunosuppression by *A. candida* could enable sex with other races and permit exchange of effector repertoires (McMullan et al., 2015). Comparative and association genomics on these different races have discovered novel class of CHxC (reclassified as CX2CX5G and abbreviated to CCG) effector family and secreted proteins (Kemen et al., 2011). We found that multiple CCG effectors are recognized by Nucleotide Binding Leucine Rich Repeat (NB-LRR) gene White Rust Resistance4 (WRR4) of *Arabidopsis*. *A. candida* strongly suppresses Toll Interleukin-1 Receptor (TIR)-NB-LRR (TNL) resistance gene-mediated defence which relies upon the Enhanced Disease Susceptibility 1 (EDS1)/Phytoalexin Deficient 4 (PAD4)/Senescence-Associated Gene101 (SAG101) complexes. Current experiments aim to functionally characterize some of the CCGs for their immunosuppression ability via the TNL mediated pathway and investigating their mechanistic role(s). Transgenic *Arabidopsis* lines expressing the candidate CCG effectors are currently being analysed for their host targets via Mass Spectrometry.

## **Effector-mediated mechanisms of plant defence evasion in *Pseudomonas syringae***

Jose S. Rufián, Diego López-Márquez, Javier Rueda, Carmen R. Beuzón & Javier Ruiz-Albert

*Instituto de Hortofruticultura Subtropical y Mediterránea, Universidad de Málaga-Consejo Superior de Investigaciones Científicas (IHSM-UMA-CSIC). Dpto.Biología Celular, Genética y Fisiología, Campus de Teatinos, Málaga, E-29071, Spain*

*Pseudomonas syringae* is a remarkably adaptive bacterial pathogen that penetrates the leaf to reach the plant apoplast, where it replicates causing disease, assuming it can counteract a two-tiered plant defense response: PTI (PAMP-Triggered Immunity, or basal resistance) and ETI (Effector-Triggered Immunity). *P. syringae* uses a type III secretion system to directly deliver effector proteins inside the plant cell cytosol, many of which are known to suppress PTI, some of which are known to trigger ETI, and a handful of which are known to suppress ETI. Bacterial infection can also trigger a plant defense response that goes beyond the local tissue, known as SAR (Systemic Acquired Resistance). We are particularly interested in the molecular and cellular mechanisms involved in effector-mediated defense evasion by *P. syringae*, in particular those involved in the suppression of ETI and SAR. Here we will present data describing *P. syringae* interference with plant immunity, by means of effector-mediated acetylation of a key positive regulator of basal, ETI, and SAR responses. Our work identifies a novel plant target for effector function, and characterizes specific residues within the plant target that are essential for its function. This work illustrates how analyzing the means by which a given effector interferes with its target can provide novel information regarding eukaryotic molecular mechanisms.



## **Exploring genetic diversity to identify virulence factors in the wheat pathogen *Zymoseptoria tritici***

Lukas Meile, Ethan Stewart, Daniel Croll, Bruce McDonald

*Plant Pathology Department, Institute of Integrative Biology, ETH Zurich, Switzerland*

*Zymoseptoria tritici* is a major pathogen of wheat and the causal agent of septoria tritici blotch. *Z. tritici* isolates exhibit remarkable differences in virulence and host specificity. Exploring the natural variation among fungal isolates provides us with outstanding information on the virulence mechanisms of fungal pathogens. By mapping quantitative trait loci (QTL), we explored the genetic bases regulating the differences in virulence of the isolates 3D7 and 3D1. Both isolates are virulent on susceptible wheat cultivars, but 3D7 induces necrosis faster and produces more pycnidia than 3D1. Although we expected quantitative differences in virulence, we identified a single QTL, which is 163 kb long and localizes in a genomic region rich in transposable elements and repetitions, indicating that it is under evolutionary pressure. Due to differences between the genomes of 3D1, 3D7 and the reference strain, manual annotation was needed and revealed 37 genes in the QTL, 4 of which were not previously annotated. De novo assembly of 3D1 and 3D7 genomes was previously performed and genomic comparison showed that the synteny in the QTL is not conserved in both parental lines. Remarkably, two major insertions, rich in transposable elements and of about 50 kb, were identified in 3D7 but not in 3D1. Interestingly, in between these two insertions we identified two genes that codify two small secreted proteins and with sequence differences between 3D7 and 3D1. We are currently investigating whether these two genes codify virulence factors of *Z. tritici*.

#### **P46: Guido SESSA**

### **The *Xanthomonas* effector XopAU is an active protein kinase that manipulates host MAP kinase signaling to promote disease**

Doron Teper, Eran Bosis, Georgy Popov, Anil Madhusoodana, and Guido Sessa

*Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv 69978, Israel*

The Gram-negative bacterium *Xanthomonas euvesicatoria* (Xe), is the causal agent of spot disease in pepper and tomato plants. Xe pathogenicity depends on a type III secretion system (T3S) that translocates effector proteins into the plant cell. We identified a novel Xe effector, XopAU, that encodes a Ser/Thr protein kinase and plays a role in Xe pathogenicity. Transient expression of XopAU in host and non-host plants promoted typical defense responses including phosphorylation of MAPKs, accumulation of pathogenesis-related (PR) proteins and elicitation of cell death. Genetic analysis of XopAU by insertional mutagenesis and overexpression revealed a role for this effector in the development of disease symptoms in pepper plants. XopAU was shown in vitro to encode a catalytically active protein kinase whose activity is required for its biological function. Protein-protein interaction studies demonstrated that XopAU physically interacts with and phosphorylates the immunity-associated MAPKK MKK2. Remarkably, Silencing of MKK2 in *N. benthamiana* reduced XopAU-mediated cell death and MAPK phosphorylation supporting the notion that MKK2 represents a target for XopAU in the host plant. Together, this study indicates that XopAU contributes to Xe disease symptoms in pepper plants by manipulating host MAPK signaling through phosphorylation of MKK2.

---

#### **P47: Alan SHULMAN**

### **Fine-mapping of the Rpt5 net blotch resistance gene region in barley**

Yaniv E<sup>1</sup>, Tanskanen J<sup>1</sup>, Törönen P<sup>1</sup>, Kalendar R<sup>1</sup>, Jalli M<sup>2</sup>, Doležel J<sup>3</sup>, Holm L<sup>1</sup>, Manninen O<sup>4</sup>, Schulman AH<sup>1,2</sup>

*1 Institute of Biotechnology, P.O. Box 65, FI-00014 University of Helsinki, Finland*

*2 Green Technology, LUKE Natural Resources Institute, FI-00790 Helsinki, Finland*

*3 Institute of Experimental Botany, CZ-78371 Olomouc, Czech Republic*

*4 Boreal Plant Breeding Ltd., FI-31600 Jokioinen, Finland*

The net blotch disease, caused by the *Pyrenophora teres* f. *teres*, is one of the most important fungal diseases of barley (*Hordeum vulgare*) in Finland. During testing of a collection of barley accessions, including landraces, for resistance to net blotch, the Ethiopian landrace CI9819 turned out to be 98% resistant, an optimal level for providing sustainable resistance. The resistance gene in CI9819 was designated Rpt5. We are using a variety of mapping strategies, including exploitation of collinearity with *B. distachyon*, to fine-map Rpt5. So far we have narrowed the Rpt5 containing segment to 0.019 cM. We have also carried out association genetics on a wide set of barley cultivars and made expression analyses of resistant and susceptible mapping parents. These approaches, combined with the genetically and physically mapped and linked barley gene space ("gene-ome") and emerging barley genome sequence assembly will greatly enhance efforts to positionally clone Rpt5.

## **Tomato R-genes are targeted by miRNA during nematode pathogenesis**

Waldemar Skowron, Magdalena wicicka, Mateusz Matuszkiewicz, Marcin Filipecki, Marek D. Koter

*Warsaw University of Life Sciences, str159 02-776 Warsaw, Poland*

Plant parasitic nematodes (PPN) evoke dramatic changes in developmental program and metabolism of selected root cells. The transcriptome dynamics during PPN infection also reflects an active suppression of defense responses. This is valid both for root knot nematodes and cyst nematodes what was shown in our previous screening of tomato genes affected during potato cyst nematode (PCN) parasitism and other studies. Among genes downregulated by nematode effectors there are classical NB-LRR R-genes, WRKY regulators and other stress related sequences. Since downregulation of tomato genes on PCN infection accounts for >30% of the total transcriptome changes, we hypothesize that miRNA mediated cleavage may play here an important role. Therefore using the microRNA-seq data we monitored the changes in micro-transcriptome with respect to defense related targets. The NRCH (Soly04g007050) is one of previously identified PCN suppressed tomato R-genes. In this research we demonstrate that NRCH is a potential target of miR159 and miR9474 present in roots. The downregulation of NRCH gene, which was documented by qRT-PCR, coincides with higher level of these miRNAs in infected roots. Such correlation was discovered in a few other cases. Moreover the microRNA-seq revealed that as much as 67% (149/221) of tomato R-genes are potentially targeted by root miRNAs which are identical or highly homologous to miRBase records ("known" miRNAs). The verification by qRT-PCR of nematode-regulated miRNAs and potential R-gene targets showed reverse correlation in the case of 14 miRNA/target transcript pairs. Mapping of cleavage sites with RACE confirmed some of postulated miRNA activities. In addition to presented data it was shown that only less than 10% of the tomato root micro-transcriptome can be defined as "known" indicating the existence of huge unexplored array of potential miRNA/R-gene interactions.

## **Characterization of *Verticillium* wilt resistance genes from *Nicotiana glutinosa* and *Humulus lupulus* reveals ancient origin of Ve1 immune receptor homologs in plants**

Yin Song<sup>1</sup>, Zhao Zhang<sup>1</sup>, Michael F. Seidl<sup>1</sup>, Branka Javornik<sup>2</sup>, and Bart P. H. J. Thomma<sup>1</sup>

*1 Laboratory of Phytopathology, Wageningen University, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands*

*2 University of Ljubljana, Biotechnical Faculty, Agronomy Department, Centre for Plant Biotechnology and Breeding, Jamnikarjeva 101, 1000 Ljubljana, Slovenia*

Pattern recognition receptors (PRRs) confer plant resistance to pathogen infection by recognizing conserved microbe-associated molecular patterns (MAMPs). The cell surface-localized receptor tomato Ve1 confers race-specific resistance against *Verticillium dahliae* by recognizing the effector Ave1 (for Avirulence on Ve1 tomato) that is secreted by race 1 strains of the soil-borne vascular wilt fungus *V. dahliae*. In addition, Ave1 homologs were found in plants and microbes, and these homologs are differentially recognized by tomato Ve1. The demonstration of interfamily transfer of tomato Ve1 to the phylogenetically distant species *Arabidopsis* implies evolutionary conservation of the underlying immune signaling cascade across plant taxonomy. Although several Ve1 homologs were identified within and outside the Solanaceae family, functionality of these homologs as immune receptor against *Verticillium* wilt often lacks. Here, we describe the cloning and characterization of *Verticillium* wilt resistance genes from *Nicotiana glutinosa* and *Humulus lupulus*, *Solanum tuberosum* and *S. torvum* as well, and demonstrate that these homologs govern resistance against *Verticillium* race 1 strains through recognition of the Ave1 effector. Phylogenetic analysis shows that Ve1 homologs are widely distributed in land plants. Our study unravels that tomato Ve1 homologs are ancestral immune receptors that are conserved across the land plant kingdom.

## **Dissection of TALE-mediated transcriptional enhancement**

Jana Streubel, Jens Boch

*Leibniz University Hannover, Institute of Plant Genetics, Herrenhäuser Straße 2, 30419 Hannover, Germany*

Plant pathogenic *Xanthomonas* bacteria translocate transcription activator-like effectors (TALEs) into host cells to specifically induce transcription of target genes. The direct binding of TALE proteins to promoter sequences is mediated by a highly conserved region that is composed of tandem 34-amino acid-repeats. These repeats mainly vary in two amino acids called repeat-variable diresidue (RVD) in which one amino acid specifically recognizes one base in the target DNA sequence<sup>1</sup>. This simple and modular architecture allows the rearrangement of repeats to generate artificial TALEs with any desired DNA-binding specificity and thereby targeted gene regulation. Although the DNA-binding mode is almost fully understood it is still ambiguous how TALE proteins activate transcription and whether additional promoter elements are needed to support efficient gene activation. Natural TALE proteins preferentially bind to core promoter regions ranging from -300 to +200bp around the transcription start site<sup>2</sup>. By using the example of the OsSWEET14 promoter we systematically positioned TALEs in proximal as well as distal promoter positions and compared their activity. Interestingly, we found that TALEs mediate robust gene activation based on diverse promoter positions although their efficiency varies. Moreover, we showed that TALEs can activate transcription by binding to the reverse strand of the OsSWEET14 promoter, which has not been described, before. Most, but not all of the analyzed TALEs shift the transcriptional start site at the OsSWEET14 promoter demonstrating a differentiated influence on transcriptional initiation. In summary, the highly position- and orientation-independent activation potential suggests that TALEs function similar to enhancer-binding proteins.

## **Identification and functional analysis of novel regulatory components of the potato NLR immune receptors Rx1 and Gpa2**

Octavina Sukarta<sup>1</sup>, Erik Slootweg<sup>1</sup>, Sijf Boeren<sup>2</sup>, Jan Roosien<sup>1</sup>, Rikus Pomp<sup>1</sup>, Jaap Bakker<sup>1</sup>, Geert Smant<sup>1</sup> and Aska Goverse<sup>1</sup>

*1 Laboratory of Nematology, Wageningen University, Wageningen, The Netherlands*

*2 Department of Biochemistry, Wageningen University, Wageningen, The Netherlands*

Following the recognition of pathogen-derived avirulence proteins, the mechanism by which plant nucleotide-binding and leucine-rich repeat (NLR) immune receptors trigger defense remains vague. However, the modular architecture of these receptors suggests that they may engage in a network of interactions with other host factors, presumably for defense signaling. Identifying interacting partners of plant NLRs is therefore key to advance our understanding of how they function. Here, we aim to identify and characterize novel regulatory components of the potato NLRs Rx1 and Gpa2. Both NLRs are characterized by an archetypical N-terminal coiled-coil (CC) domain and share high sequence conservation. Nonetheless, they mediate distinct responses against two unrelated pathogens, providing a unique platform for research. Rx1 confers rapid, extreme resistance against Potato Virus X (PVX) whilst Gpa2 triggers mild resistance against the potato cyst nematode *Globodera pallida*. Existing models predict the N-terminal domain to act as a platform for downstream interactions. We therefore used the CC domains of Rx1 or Gpa2 as baits in a Co-IP/MS analysis to co-purify putative interactors from *Nicotiana benthamiana*. Five hits (designated Rp01-Rp05) were further prioritized as candidate Rx1/Gpa2 interacting proteins. Similar pull-down experiments corroborated complex formation with the full-length immune receptors in planta. A combination of reverse genetics and advanced microspectroscopic studies was then used to resolve the functional relevance of the interactions detected. Interestingly, we could demonstrate that co-expression of Rp05 alters the subcellular distribution of the Rx1-CC domain, hinting that Rp05 may be involved in Rx1-functioning. It is also worth noting that transient overexpression of Rp05 enhanced resistance against PVX independently of Rx1, pointing to its importance as a component in immune signaling. We currently focus on substantiating this model by investigating the broader role of Rp05 in defense against other pathosystems.

## **Novel tomato miRNAs are the predominant component of RNAi upon *Globodera rostochiensis* infection**

Magdalena Swiecicka, Waldemar Skowron, Piotr Cieszyński, Marcin Filipecki, Marek D. Koter

*Warsaw University of Life Sciences, Nowoursynowska str. 159, 02-776 Warsaw, Poland*

Many studies suggest the role of miRNA in plant response to diverse pathogens and herbivores. In our study we analyzed the dynamics of miRNAs in *Globodera rostochiensis* infected tomato roots 3, 7 and 10 days post inoculation. Using bioinformatic tools we detected differentially expressed known miRNAs (described in miRBase), as well as many more sequences belonging to new miRNA species. The analyzes were done based on Illumina HiScanSQ sequenced libraries prepared in three replicates giving over 10 M reads per each replicate. sRNAWorkbench software package was used for discovery of known (miRProf) and new (miRCat) microRNA sequences. Quantitative sequence data were analyzed using edgeR and DESeq tools. miRNAs with the largest fold change -5 to 4 (log2FC) between infected and control samples were used for discovery of their target sequences with the help of psRNATarget. Totally 122 known miRNAs were identified (identical or highly similar to sequences deposited in miRBase). Most of them were represented by numerous variants known as isomiRs. miRNA diversity was greatly extended by over 400 newly discovered miRNAs possibly playing a role in plant-nematode interaction. The qPCR confirmed expression changes in approx. 25% of miRNA candidates. About 25% of detected miRNA showed up-regulation, whereas 33% down-regulation at any of 3 time points. About 42% of miRNAs show mixed profiles and are up- and down-regulated at different infection stages. The putative targets represent a wide range of functional categories including 457 transcription factors (including 27 WRKY, 50 bZIP), 289 genes encoding proteins with LRRs and 877 kinases (14 receptor kinases) possibly involved in responses to nematode parasitism. Besides known and unknown miRNAs the micro-transcriptome is composed in great majority of other sequences (eg. siRNA, ta-siRNA, tRFs). The regulatory potential of this transcriptome component is discussed.

## **Functional analysis of the TALome of african *Xanthomonas oryzae* pv. *oryzae* reveals a new bacterial leaf blight susceptibility gene candidate**

Tu Tran Tuan<sup>1</sup>, Alvaro Pérez-Quintero<sup>1</sup>, Mathilde Hutin<sup>1</sup>, Jan Leach<sup>2</sup>, Valérie Verdier<sup>1,2</sup>, Sébastien Cunnac<sup>1</sup>, Ralf Koebnik<sup>1</sup>, Boris Szurek<sup>1</sup>

*1 UMR Interactions Plantes Microorganismes Environnement, IRD-Cirad-UM, Montpellier, France*

*2 Bioagricultural Sciences and Pest Management, Colorado State University, FortCollins, USA*

Bacterial plant pathogenic *Xanthomonas* translocate Transcription Activator-Like (TAL) effectors into plant cells to function as specific plant transcription factors via a novel programmable DNA-binding domain. Rice-pathogenic *Xanthomonas oryzae* pv. *oryzae* (Xoo) strains contain multiple TAL genes varying from 9 to 16 in African and Asian strains respectively. While one or two act as major virulence factors, the relative contribution of the other members to Xoo pathogenicity remains unclear. To address that question, we systematically analyzed the function of each of the nine TAL effectors of African Xoo strains MAI1 and BAI3. TAL genes were directly sub-cloned into an expression vector suitable for functional analysis from a cosmid genomic DNA library. Sequencing of the repeat region shows that both TALomes are highly similar and include seven conserved TAL effector clusters. TAL effector-deficient Xoo strains X11-5A carrying each single TAL effector gene from both strains were assessed for gain of virulence on susceptible rice. At least four TAL effectors provided increased virulence to X11-5A, including three TAL effectors known to target the susceptibility S gene SWEET14. Using programs to predict for TAL targets, a fourth TAL effector was reported to target a new S gene candidate. Our most recent data on the functional analysis of this new major virulence TAL effector and its targets will be presented.



## **How plant pathogenic bacteria co-opt the ubiquitin-proteasome system**

Suayib Üstün<sup>1</sup>, Arsheed Sheikh<sup>3</sup>, Alex Jones<sup>3</sup>, Wolfgang Hoehenwarter<sup>4</sup>, Vardis Ntoukakis<sup>3</sup> and Frederik Börnke<sup>1,2</sup>

*1 Leibniz-Institute for Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany*

*2 Institute for Biochemistry and Biology, University of Potsdam, Germany*

*3 School of Life Sciences University of Warwick, Coventry, United Kingdom*

*4 Leibniz Institute for Plant Biochemistry, Halle (Saale), Germany*

Plant pathogenic bacteria translocate about 30 type-III effector proteins (T3E) into the host cell to cause disease. These T3Es manipulate processes including secretion, the ubiquitin-proteasome system (UPS) and gene expression. Evidence is emerging that manipulation of the UPS might be an effective and widespread virulence strategy of bacterial invaders to promote pathogenesis. In line with this, we could show that *Xanthomonas* T3E XopJ promotes virulence through the inhibition of the proteasome and a resultant suppression of SA-dependent defense. XopJ acts as a cysteine protease to degrade proteasomal subunit RPT6 triggering proteasome malfunction. Consequently, XopJ-mediated suppression of the proteasome impairs the proteasomal turnover of NPR1 leading to its accumulation. Preliminary analysis of the XopJ-induced ubiquitylome revealed candidates implicated in UPS, vesicle trafficking and calcium signalling. In addition, we show that *Pseudomonas syringae* also inhibits proteasome activity in a type-III secretion dependent manner. A systematic screen for T3Es from *Pseudomonas* for their ability to interfere with proteasome activity revealed HopM1, HopAO1 and HopG1 as candidates. Identification of proteins interacting with HopM1 by mass-spectrometry indicate that HopM1 resides in a complex together with several E3 ubiquitin ligases and proteasome subunits, supporting the observation and hypothesis that HopM1 is ubiquitylated in plants to associate with the proteasome leading to its inhibition. Further functional characterization of other *Xanthomonas* T3Es unveiled effectors localized in the nucleus that interact with UPS components to stabilize transcription factors. Thus, the manipulation of the host cell proteasome is an efficient virulence mechanism of phytopathogens that evolved different effector repertoires.

## **HpaP modulates type 3 effector secretion in *Ralstonia solanacearum* and plays an essential role in virulence**

David Lohou<sup>1,2</sup>, Marie Turner<sup>1,2</sup>, Fabien Lonjon<sup>1,2</sup>, Anne-Claire Cazalé<sup>1,2</sup>, Nemo Peeters<sup>1,2</sup>, Stéphane Genin<sup>1,2</sup> and Fabienne Vailleau<sup>1,2,3</sup>

1 INRA, Laboratoire des Interactions Plantes Micro-organismes (LIPM), UMR441, Castanet-Tolosan, France

2 CNRS, Laboratoire des Interactions Plantes Micro-organismes (LIPM), UMR2594, Castanet-Tolosan, France

3 Université de Toulouse; INP; ENSAT; 18 chemin de Borde Rouge, 31326 Castanet-Tolosan, France

The Gram-negative bacterium *Ralstonia solanacearum*, the causal agent of bacterial wilt, is a worldwide major crop pest whose virulence strongly relies on a type 3 secretion system (T3SS). This extracellular apparatus allows the translocation of proteins, called type 3 effectors (T3Es), directly into the host cells. To date, very few data are available in *R. solanacearum* concerning the role played by type 3-secretion regulators at a post-translational level. With this work, we demonstrate that HpaP, a putative type 3 secretion substrate specificity switch (T3S4) protein of *R. solanacearum*, which is not secreted/translocated by the bacterium, controls T3E secretion in *R. solanacearum*. We showed that HpaP modulates the secretion of early (HrpY pilin) and late (AvrA and PopP1 T3Es) type 3 substrates, and interacts with the PopP1 T3E. We also identified two regions of five amino acids in the T3S4 domain that are essential for efficient PopP1 secretion and for HpaP's role in virulence on tomato and *Arabidopsis thaliana*. Thus, HpaP is a putative *R. solanacearum* T3S4 protein important for full pathogenicity on several hosts, acting as a helper for PopP1 secretion, and repressing AvrA and HrpY secretion.

## **The receptor-like kinase SOBIR1/EVR is essential for immune signalling downstream of Cf-4**

Aranka M. van der Burgh<sup>1</sup>, Guozhi Bi<sup>1,2</sup>, Thomas W. H. Liebrand<sup>1,3</sup>, Matthieu H.A.J. Joosten<sup>1</sup>

*1 Laboratory of Phytopathology WUR, Droevendaalsesteeg 1, 6708 PB Wageningen, The Netherlands*

*2 College of Horticulture, Northeast Agricultural University, Harbin, PR China*

*3 Current address Department of Plant Pathology 576 Hutchison Hall, University of California Davis, USA*

Receptor-like proteins (RLPs) are cell surface receptors that perceive microbial patterns present in the apoplast. RLPs in most cases carry extracellular leucine-rich repeats (LRRs), but lack an intracellular kinase domain for activation of downstream signaling upon ligand perception. Recently, we showed that tomato (*Solanum lycopersicum*) Cf-4, an LRR-RLP mediating resistance to Avr4-expressing strains of the fungal pathogen *Cladosporium fulvum*, constitutively interacts with the receptor-like kinase (RLK) SUPPRESSOR OF BIR-1/EVERSHED (SOBIR1/EVR). Interestingly, it was found that SOBIR1 is involved in signalling downstream of Cf-4, for which its kinase domain is essential. We also found that the Cf-4/SOBIR1 complex recruits the RLK BAK1 upon its activation by Avr4. We hypothesise that the kinase domain of the Cf-4/SOBIR1 complex is trans-phosphorylated by BAK1 upon its recruitment and currently we are examining SOBIR1 phosphorylation using several approaches. Firstly, to determine whether SOBIR1 is differentially phosphorylated, we are performing GFP pull-downs from transgenic *Nicotiana benthamiana* plants expressing Cf-4-eGFP, in the presence or absence of Avr4. We are analysing the phosphorylation status of co-purifying SOBIR1 that is associated with Cf-4-eGFP. Secondly, a tyrosine residue might be the 'gatekeeper' for phosphorylation of the kinase domain of SOBIR1. To test this, we individually mutated the eight tyrosine residues of the AtSOBIR1 kinase domain to phenylalanine residues. These mutants were screened for loss of constitutive cell death induction in *N. tabacum*, which is known to require a functional kinase domain. Preliminary data suggest that single mutation of Y436 and Y538 reduces the ability of AtSOBIR1 to induce cell death.

## **Two R-genes at a single genetic locus confer resistance to Clubroot in Oilseed Rape**

Michel Van Thournout<sup>1</sup>, Vanessa Hostyn<sup>1</sup>, Kim Crommar<sup>1</sup>, Godfrey Chongo<sup>2</sup>, Marc Bots<sup>1</sup>, Stephen Rae<sup>1</sup>, Jean Broadhvest<sup>1</sup> and Steven Engelen<sup>1</sup>

*1 Bayer CropScience NV, Technologiepark 38, 9052 Ghent, Belgium*

*2 Bayer CropScience Inc, Site 600, Box 117, R.R. #6, Saskatoon, Canada*

Clubroot, a disease caused by the protist *Plasmodiophora brassicae*, impacts significantly the production of oilseed rape (*Brassica rapa* and *Brassica napus*) and other Crucifer species. Clubroot infections cause a characteristic severe swelling of the roots leading to delayed flowering, wilting, leaf yellowing, severe root gall and premature ripening. *P. brassicae* spreads via spores that are viable for several years in the soil, therefore limiting the cultivation of susceptible crops in infested fields. An effective way to manage this disease is deployment of disease resistance genes found in different Brassica species. Here we describe the map based cloning of a *B. napus* genetic region that confers resistance to Clubroot. cDNA sequencing was performed to improve the annotation of the disease resistance locus on the genomic reference sequence of *B. napus*. The genetic locus comprised 2 genes containing CC-NBS-LRR domains. Loss-of-function mutants indicate that both genes contribute to the resistant phenotype. How they interact remains to be elucidated.

## PARTICIPANTS

AGUILERA	Carolina	carolina.aguileragalvez@wur.nl	The Netherlands
ALBERS	Philip	albers@igzev.de	Germany
ALMEIDA	Nuno	nalmeida@itqb.unl.pt	Portugal
BALLINI	Elsa	ballini@supagro.inra.fr	France
BAUTERS	Lander	lander.bauters@ugent.be	Belgium
BINDICS	Janos	jbindics@gmail.com	Austria
BOCH	Jens	jens.boch@genetik.uni-hannover.de	Germany
BONAS	Ullas	ulla.bonas@t-online.de	Germany
BOSIS	Eran	bosis@post.tau.ac.il	Israël
BOURRAS	Salim	s.bourras@botinst.uzh.ch	Switzerland
BOUWMEESTER	Klaas	klaas.bouwmeester@wur.nl	The Netherlands
CANADO	Audrey	audrey.canado@supagro.inra.fr	France
COCKRAM	James	james.cockram@niab.com	United Kingdom
COLL	Anna	anna.coll@nib.si	Slovenia
CROLL	Daniel	daniel.croll@usys.ethz.ch	Switzerland
DAGDAS	Yasin	yasin.dagdas@tsl.ac.uk	United Kingdom
DE GUILLEN	Karine	karine.deguillen@cbs.cnrs.fr	France
DIAZ GRANADOS	Amalia	amalia.diazgranadosmunoz@wur.nl	The Netherlands
DJAMEI	Armin	armin.djamei@gmi.oeaw.ac.at	Austria
DOWNIE	Rowena	rcd40@cam.ac.uk	United Kingdom
DREISEITL	Antonin	dreiseitl.antonin@vukrom.cz	Czech Republic
ELASHRY	Abdelnaser	elashry@uni-bonn.de	Germany
ENGELN	Steven	steven.engelen@bayer.com	Belgium
ERCOLANO	Maria Raffaella	ercolano@unina.it	Italia
ESCHEN LIPPOLD	Lennart	leschen@ipb-halle.de	Germany
EVARD	Alexandre	alexandre.evrand@vilmorin.com	France
EVES VAN DEN AKKER	Sebastian	s.evesvandenakker@dundee.ac.uk	United Kingdom
FAVERY	Bruno	favery@sophia.inra.fr	France
FILIPECKI	Marcin	marcin_filipecki@sggw.pl	Poland
FONDEVILLA	Sara	sfondevilla@ias.csic.es	Spain
GARRIDO	Sharon	sb_garrido@yahoo.com	Germany
GEFFROY	Valérie	valerie.geffroy@ips2.universite-paris-saclay.fr	France
GHEYSEN	Lieve	Godelieve.gheysen@UGent.be	Belgium
GIBRIEL	Hesham	hesham.gibriel@wur.nl	The Netherlands
GODIARD	Laurence	laurence.godiard@toulouse.inra.fr	France
GOVERSE	Aska	aska.goverse@wur.nl	The Netherlands
HOSER	Rafal	rhoser@ibb.waw.pl	Poland
IOVINO	Paolo	paolo.iovino@gmail.com	Italia
JONES	John	john.jones@hutton.ac.uk	United Kingdom
JOOSTEN	Matthieu	Matthieu.Joosten@wur.nl	The Netherlands
JAOUANNET	Maele	maele.jaouannet@hutton.ac.uk	United Kingdom
KAHMANN	Regine	kahmann@mpi-marburg.mpg.de	Germany

KAMOUN	Sophien	sophien.kamoun@tsl.ac.uk	United Kingdom
KNIP	Marijn	m.knip@uva.nl	The Netherlands
KOEBNIK	Ralf	koebnik@gmx.de	France
KRASILEVA	Ksenia	Ksenia.Krasileva@tgac.ac.uk	United Kingdom
KROJ	Thomas	thomas.kroj@supagro.inra.fr	France
KRZYMOWSKA	Magdalena	krzyna@ibb.waw.pl	Poland
KWIATKOWSKI	Jakub	jagerinfantry@gmail.com	Poland
LEBRUN	Marc-Henri	marc-henri.lebrun@versailles.inra.fr	France
LIBANTOVA	Jana	jana.libantova@savba.sk	Slovakia
LILLEMO	Morten	morten.lillemo@nmbu.no	Norway
LIN	Xiao	xiao.lin@wur.nl	The Netherlands
LONJON	Fabien	fabien.lonjon@toulouse.inra.fr	France
LOPÉZ MARQUEZ	Diego	dIm@uma.es	Spain
MAEKAWA	Takaki	maekawa@mpipz.mpg.de	Germany
MANTELIN	Sophie	sophie.mantelin@hutton.ac.uk	United Kingdom
MAQBOOL	Abbas	abbas.maqbool@jic.ac.uk	United Kingdom
MERDA	Déborah	deborah.merda@angers.inra.fr	France
MARTIN	Francis	fmartin@nancy.inra.fr	France
MARTIN	Gregory	gbm7@cornell.edu	U.S.A
MISAS VILLAMIL	Johana c	jmisas@uni-koeln.de	Germany
MISSONNIER	Hélène	helene.missonnier@syngenta.com	France
MOREL	Alice	alice.morel@toulouse.inra.fr	France
MOREL	Jean-Benoit	jbmorlet@cirad.fr	France
MORLET	Claire	claire.morlet@syngenta.com	France
NAALDEN	diana	diana.naalden@gmail.com	Belgium
NEEMA	Claire	Claire.neema@supagro.inra.fr	France
NOEL	Laurent	laurent.noel@toulouse.inra.fr	France
ORTIZ	Diana	diana.ortiz@supagro.inra.fr	France
PALMA GUERRERO	Javier	javier.palma@usys.ethz.ch	Switzerland
PEETERS	Nemo	nemo.peeters@toulouse.inra.fr	France
PIDON	Hélène	helene.pidon@ird.fr	France
PLANAS	Marc	marc.planas@cragenomica.es	Spain
PRATX	Loris	loris.pratx@sophia.inra.fr	France
PRUSKY	Dov	dovprusk@volcani.agri.gov.il	Israël
PYLYPENKO	Liliya	liliya.pylypenko@gmail.com	Ukraine
QUENTIN	Michael	michael.quentin@sophia.inra.fr	France
RAATS	Dina	Dina.Raats@tgac.ac.uk	United Kingdom
REDKAR	Amey	amey.redkar@tsl.ac.uk	United Kingdom
RESCHKE	Maik	maik.reschke@genetik.uni-hannover.de	Germany
RUIZ ALBERT	Javier	javieruizal@uma.es	Spain
SANCHEZ COLL	Núria	nuria.sanchez-coll@cragenomica.es	Spain
SANCHEZ VALLET	Andrea	andreasanchezvallet@gmail.com	Switzerland
SCHIRAWSKI	Jan	jan.schirawski@rwth-aachen.de	Germany
SCHULMAN	Alan	alan.schulman@helsinki.fi	Finland
SESSA	Guido	guidos@post.tau.ac.il	Israël

SKOWRON	Waldemar	waldemar_skowron@sggw.pl	Poland
SONG	Yin	yin.song@wur.nl	The Netherlands
STRAVIDOU	Ioanna	ioanna.stavridou@cost.eu	Belgium
STREUBEL	Jana	jana_streubel@gmx.de	Germany
STUKENBROCK	Eva	estukenbrock@bot.uni-kiel.de	Germany
SUKARTA	Octavina	octavina.sukarta@wur.nl	The Netherlands
SWIECICKA	Magdalena	magdalena_swiecicka@sggw.pl	Poland
SZUREK	Boris	boris.szurek@ird.fr	France
TAKKEN	Frank	f.l.w.takken@uva.nl	The Netherlands
THARREAU	Didier	tharreau@cirad.fr	France
THOMMA	Bart	bart.thomma@wur.nl	The Netherlands
TINTOR	Nico	N.Tintor@uva.nl	The Netherlands
TOKTAY	Halil	toktay@yahoo.com	Turkey
USTUN	Suayib	uestuen@igzev.de	Germany
VAILLEAU	Fabienne	fabienne.vailleau@toulouse.inra.fr	France
VALLS	Marc	marcvalls@ub.edu	Spain
VAN DER BURGH	Aranka	aranka.vanderburgh@wur.nl	The Netherlands
VAN DER DOES	Charlotte	h.c.vanderdoes@uva.nl	The Netherlands
VAN DER HOORN	Renier	renier.vanderhoorn@plants.ox.ac.uk	United Kingdom
VAN THOURNOUT	Michel	michel.vanthournout@bayer.com	Belgium
VLEESHOUWERS	Vivianne	Vivianne.Vleeshouwers@wur.nl	The Netherlands

