4th Annual Conference of the COST Action

1st – 3rd March 2017, Bled, Slovenia

PROGRAM

&

ABSTRACTS BOOK

Agricultural Institute of Slovenia

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Conference overview

Registration: Tuesday 18:30 – 20:00 and Wednesday 7:30 – 8:30

Wednesday 1st March	Thursday 2nd March	Friday 3rd March
8:30 - 8:45 Welcome		
8:45 - 10:25 Session 1 (WG1) Effectors and Virulence	**8:30 - 10:00** Session 3 (WG4) *R* Genes and Effector-Informed Resistance	**8:30 - 10:00** Session 4 (WG2) Plant proteins and processes targeted by effectors
Break	**Break**	**Break**
10:55 - 12:15 Session 1 (WG1) Effectors and Virulence	**10:30 - 12:00** Session 3 (WG4) *R* Genes and Effector-Informed Resistance	**10:30 - 12:00** Session 4 (WG2) Plant proteins and processes targeted by effectors
		12:00 - 12:15 Concluding Remarks
12:30 - 14:00 Lunch	**12:00 - 13:30** Lunch	**12:30-14:00** Lunch
14:00 – 14:30 Wenbo MA Invited lecture	**13:45 - 16:15** Visit of Castle “Blejski grad”	
14:30 -15:30 Session 2 (WG3) Effector evolution and diversification	**Break**	
Break	**16:45- 17:45** Presentation of Activities of the Sustain Action	
16:00 - 17:30 Session 2 (WG3) Effector evolution and diversification	**17:45 - 18:45** MC Meeting	
18:15 - 19:15 Poster Session 1	**18:00 - 19:30** Poster Session 2	
19:45 Dinner	**20:00** Gala Dinner	

COST FA1208

Pathogen-informed strategies for sustainable broad-spectrum crop resistance

1st – 3rd March 2017, Bled, Slovenia

Organizers

Barbara Gerič Stare	Agricultural Institute of Slovenia
Thomas Kroj	INRA, UMR BGPI, Montpellier, France
Saša Širca	Agricultural Institute of Slovenia

Scientific Committee

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Aska Goverse	Wageningen University, the Netherlands
Branka Javornik	University of Ljubljana, Slovenia
Beat Keller	University of Zurich, Switzerland
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Nemo Peeters	INRA, Toulouse, France
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Bart Thomma	Wageningen University, the Netherlands
Hans Thordal-Christensen	University of Copenhagen, Denmark
Didier Tharreau	CIRAD, Montpellier, France
Vivianne Vleeshouwers	Wageningen University, the Netherlands

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TABLE OF CONTENTS

SCIENTIFIC PROGRAM

WEDNESDAY 1ST OF MARCH

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

SESSION 1 PATHOGEN EFFECTORS AND VIRULENCE
CHAIR: Lieve GHEYSEN & Sasa SIRCA

8:45-9:05 **Armin DJAMEI** ***(Gregor Mendel Institute, AU)***
Ustilago maydis effectors targeting plant hormone pathways

9:05-9:25 **Alexandra PELGROM** ***(Utrecht University, NL)***
Effectors and their plant targets as leads for downy mildew resistance breeding in lettuce

9:25-9:45 **Helena VOLK** ***(University of Ljubljana, SI)***
Assigning function to recombinant effector proteins implicated in *Verticillium* wilt of hops

9:45-10:05 **Abdelnaser ELASHRY** ***(University of Bonn, DE)***
Identification of new putative effectors by investigating *Heterodera schachtii* transcriptome

10:05-10:25 **Boris SZUREK** ***(IRD, Montpellier, FR)***
Functional Analysis of The TALome of African *Xanthomonas oryzae pv. oryzae* Reveals a New Bacterial Leaf Blight Susceptibility Gene Candidate

10:25-10:55 BREAK

10:55-11:15 **Malaika EBERT** ***(Wageningen University, NL)***
Transfer of fungal-derived toxin tolerance to crop plants to engineer resistance to *Cercospora* diseases

11:15-11:35 **Fabienne VAILLEAU** ***(INRA,Toulouse, FR)***
How type 3-associated proteins control *Ralstonia solanacearum* species complex pathogenicity?

11:35-11:55 **Heike PROCHASKA** ***(Martin Luther University Halle-Wittenberg, DE)***
Mechanistic aspects of type III-dependent translocation of *Xanthomonas* effector proteins

11:55-12:15 **Sebastian EVES-VAN DER AKKER** ***(University of Dundee and John Innes Centre, UK)***
Genomics, effectors and resistance against plant-parasitic nematodes

12:30-14:00 LUNCH

14:00 – 14:30 **Invited Lecture: Wenbo MA** ***(Riverside, USA)***
Phytophthora effectors promote infection by suppressing small RNA silencing in plants

SESSION 2 EFFECTOR EVOLUTION AND DIVERSIFICATION
CHAIRS: Didier THARREAU & Barbara GERIC STARE

14:30-14:50 **Laurent NOËL** ***(INRA Toulouse, FR)***
Xanthomonas campestris Tal effector repertoire evolved from two ancestral genes

14:50-15:10 **Krzysztof PAWŁOWSKI** ***(Warsaw University, PL)***
Novel families of pathogen effector kinases in plants. Evolutionary origin and biological function

15:10-15:30 **Lionel GAGNEVIN** ***(CIRAD, Montpellier, FR)***
Microevolution of *TAL* effector genes in *Xanthomonas citri* pv. *citri* during a Citrus canker epidemic

15:30-16:00 BREAK

16:00-16:20 **Peter THORPE** ***(The James Hutton Institute, Dundee, UK)***
Genome and RNA sequencing of agriculturally important aphid species reveals conserved and divergent effector sets and host specific effector deployment

16:20-16:40 **Johana MISAS VILLAMIL** ***(University Of Cologne, DE)***
Host adaptation of the fungal effector pit2: a case of multiple evolutionary events

16:40-17:00 **Remco STAM** ***(Technical University of Munich, DE)***
Using population genomics to understand NLR evolution in wild tomato

17:00-17:30 **Didier THARREAU** ***(CIRAD, Montpellier, FR)***
What have we learnt on the evolution of avirulence genes?
A spotlight on fungal pathogens with particular emphasis on *Magnaporthe oryzae*

18:15-19:15 **POSTER SESSION 1** *First 30 minutes odd n°, second 30 min pair n°*

19:45 DINNER

THURSDAY 2ND OF MARCH

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

CHAIRS: Vivianne VLEESHOUWERS & Beat KELLER

8:30-8:50 **Stella CESARI (*INRA, Montpellier, FR*)**
Cytosolic activation of cell death and stem rust resistance by homomeric cereal MLA-family CC-NLR proteins

8:50-9:10 **Aranka VAN DER BURGH *(Wageningen University, NL)***
SOBIR1-mediated immunity requires conserved tyrosine residues in its kinase domain

9:10-9:30 **Magdalena KRZYMOWSKA *(Institute of Biochemistry and Biophysics PAS, Warsaw, PL)***
Regulatory role of the truncated isoform of tobacco N receptor

9:30-10:00 **Vivianne VLEESHOUWERS *(Wageningen University, NL)***
Effector-driven breeding for disease resistance in potato

10:00-10:30 BREAK

10:30-10:50 **Erik SLOOTWEG *(Wageningen University, NL)***
Inter- and intramolecular interactions regulating the activity of the CNL immune receptors Rx1 and Gpa2 in complex with RanGAP2

10:50-11:10 **Tzion FAHIMA (*University of Haifa, IL*)**
Evolution and adaptation of wild emmer populations to wheat pathogens

11:10-11:30 **Chih-Hang WU *(The Sainsbury Laboratory, UK)***
A complex NLR signaling network mediates immunity to diverse plant pathogens

11:30-12:00 **Beat KELLER *(University of Zurich, CH)***
Host and Pathogen-Informed Strategies to Achieve Durable Resistance in Cereals

12:00-13:30 LUNCH

13:45-16:15 Visit of Castle "Blejski grad"

16:15-16:45 BREAK

16:45-17:45 **SUSTAIN COST ACTION NETWORK**: PRESENTATION OF ACTIVITIES OF THE SUSTAIN ACTION & PRESENTATION OF STSM BY LIEVE GHEYSEN & STSM PRESENTATIONS
17:45-18:45 MANAGEMENT COMMITTEE MEETING *(Management committee members only)*

18:00-19:30 **POSTER SESSION 2** *First 45 minutes pair n°, second 45 min odd n°*

20:00 GALA DINNER

FRIDAY 3RD OF MARCH

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

CHAIRS: Hans THORDAL-CHRISTENSEN, Anna COLL & Nemo PEETERS

8:30-8:50 Mark BANFIELD *(John Innes Centre, UK)*
Interactions between rice blast effectors and rice HMA domains that underpin disease resistance or susceptibility

8:50-9:10 Frederik BORNKE *(Leibniz-Institute for Vegetable and Ornamental Crops,DE)*
Co-option of the host ubiquitin-proteasome system by plant pathogenic bacteria

9:10-9:30 Tolga Osman BOZKURT *(Imperial College London, UK)*
RXLR effector PexRD54 employs host components to stimulate biogenesis and relocation of autophagosomes toward haustoria

9:30 -10:00 Hans THORDAL-CHRISTENSEN *(University of Copenhagen, DK)*
Effort to uncover powdery mildew fungal effectors and their targeting of host processes

10:00-10:30 BREAK

10:30-10:50 Anna COLL *(National Institute of Biology, Ljubljana, SL)*
Novel Crosstalk Between Ethylene And Salicylic Acid Signalling Pathways In Virus Infected Potato Unravelled By Network Analysis

10:50-11:10 Sebastian BECKER *(Leibniz University Hannover, DE)*
Flexibility in tales – an adaption to variable targets and plant resistance

11:10-11:30 Georgy POPOV *(Tel Aviv University, IL)*
The *Xanthomonas* type III effector XopAE encodes an E3 ubiquitin ligase that inhibits PAMP-triggered immunity

11:30-12:00 Nemo PEETERS *(INRA, Toulouse, FR)*
A selection of my favourite effector-stories from the last years

12:00-12:15 CONCLUDING REMARKS AND CLOSURE OF THE CONFERENCE

12:30-14:00 LUNCH

ABSTRACTS OF ORAL PRESENTATIONS

Wednesday 1st of March 2017

Session 1

PATHOGEN EFFECTORS AND VIRULENCE (WG1)

Armin DJAMEI

Ustilago maydis effectors targeting plant hormone pathways

Janos Bindics*, Simon Uhse*, Fernando Navarrete*, Michelle Gallei*, Armin Djamei*

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

Biotrophic plant pathogenic fungi employ a battery of small secreted molecules, so called effectors, to suppress host defense responses and to redirect the host metabolism in favor of the invader. Although effector proteins are shaping the interaction between the pathogen and the host, it is challenging to elucidate their function as they largely show no sequence homology to proteins with known functional domains. The maize infecting tumor inducing fungus *Ustilago maydis* became in the past decades a model to study biotrophic interactions. In a systematic approach we are studying the involvement of *U. maydis* effectors in hormone signaling. Here we report about the progress in elucidating new effector functions in the smut fungus *UstilJago maydis*. We present a new group of effectors that target a conserved hormonal signaling pathway in the host plant maize and describe their influence on virulence, their place of action, the identification of the host sided targets and their possible application as a tool for plant biologists.

Alexandra PELGROM

Effectors and their plant targets as leads for downy mildew resistance breeding in lettuce

Alexandra Pelgrom[1], Joyce Elberse[1], Thijs Koorman[2], Mike Boxem[2], Guido Van den Ackerveken[1]

[1] *Plant-Microbe Interactions, and* [2]*Developmental Biology, Department of Biology, Utrecht University, The Netherlands*

Infection of susceptible lettuce plants by the downy mildew pathogen *Bremia lactucae* leads to major crop losses. Protection provided by classical resistance genes is usually rapidly broken by constantly evolving races of *Bremia*. Hence, there is a growing need for more durable alternatives in resistance breeding. Downy mildews, as other oomycetes, translocate effectors, e.g. RXLR proteins, into plant cells. Effectors are best known for their suppression of plant immunity by modifying and interfering with cellular host processes, thereby allowing for and promoting infection. Although RXLR effectors are ubiquitously encoded in oomycete genomes, the plant targets of only a few are known. Insight into the molecular mechanisms underlying effector-mediated suppression of immunity may provide new leads for resistance breeding. In our research project we have identified candidate plant targets of *Bremia* effectors using the yeast-two-hybrid (Y2H) system. A set of 47 previously described as well as newly discovered *Bremia* effectors was screened against a lettuce cDNA library. For 22 effectors lettuce targets were identified. The vast majority of interactions is between a single effector and lettuce target, although up to five effectors were found to interact with a single lettuce target. A subset of effector-target interactions is further investigated by microscopic and biochemical methods. Lettuce RNAi lines are under construction to validate the biological significance of the identified effector targets.

Helena VOLK

Assigning function to recombinant effector proteins implicated in *Verticillium* wilt of hops

Helena Volk[a], Sabina Berne[a], Kristina Marton[a], Vasja Progar[a], Marko Dolinar[b], Branka Javornik[a]

[a] *Department of Agronomy, Biotechnical Faculty, University of Ljubljana, Slovenia*
[b] *Department of Chemistry and Biochemistry, Faculty of Chemistry and Chemical Technology, University of Ljubljana, Slovenia*

Verticillium wilt of hops is a vascular plant disease predominantly caused by a hemibiotrophic sordariomycete *Verticillium nonalfalfae* (*Vna*). Unravelling effectors' mode of action may reveal yet unknown processes involved in host defence or mechanisms essential for *V. nonalfalfae* virulence.The present study concerns two effector proteins *Vna*SSP4.2 and *Vna*CBP8.213 that are highly expressed during infection of hop and implicated in disease progression or modulation of host physiology. *Vna*SSP4.2 is a *Verticillium*-specific small basic protein with a predicted signal peptide and a prokaryotic membrane lipoprotein lipid attachment site. *Vna*CBP8.213 is a carbohydrate binding protein with six hevein domains.RNA-Seq and RT-qPCR gene expression profiling showed that the abundance of both transcripts significantly increased over time in roots and shoots of a susceptible hop cultivar. In a resistant hop, the expression of *VnaSSP4.2* was low and observed at 6 dpi only in roots, peaking at 12 dpi in roots and 18 dpi in shoots, and declining at later time points. The expression of *Vna*CBP8.213 in the resistant cultivar was low, observed primarily in roots and peaked at 18 dpi. Purified recombinant *Vna*SSP4.2, produced in *E. coli* BL21(DE3)pLysS with N-terminal His_6 tag, bound to multilamellar lipid vesicles in sedimentation assays. Moreover, *Vna*SSP4.2 formed SDS- and DTT-resistant dimers in POPC:PG, POPC:PE, POPC:SM and POPC:PS vesicles. Surface plasmon resonance experiments are underway to determine lipid binding kinetics and affinity. Purified recombinant *Vna*CBP8.213 has been produced in *E. coli* SHuffle cells and will be tested for chitin binding, induction of ROS and HR in host and model plants.

Abdelnaser ELASHRY

Identification of new putative effectors by investigating *Heterodera schachtii* transcriptome

Abdelnaser M. Elashry[1], Samer S. Habash[2], Nahal Ahmadinejad[3], Heiko Schoof[4], Florian M.W.Grundler[5]

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[2] *INRES Molecular Phytomedicine, University of Bonn, Karlrobert-Kreiten-Str.13, Bonn 53115, Germany*
[3] *INRES Crop Bioinformatics, University Bonn, Katzenburgweg 2, 53115 Bonn, Germany*
[4] *INRES Crop Bioinformatics, University Bonn, Katzenburgweg 2, 53115 Bonn, Germany*
[5] *INRES Molecular Phytomedicine, University of Bonn, Karlrobert-Kreiten-Str. 13, 53115 Bonn, Germany*

Beet cyst nematodes (Heterodera schachtii) 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 H. schachtii and its host, the identification of H. schachtii effectors is a crucial step. Aiming to identify H. schachtii putative effectors, we sequenced its transcriptome using next generation sequencing. Analysing the resulted sequences led us to identify a subset of sequences representing the putative secretory proteins. Comparing of the identified subset with esophageal gland-related cyst- and root-knot nematodes resulted in identifying a subset of esophageal gland related sequences and common putative effectors across the tested species. Structural and functional annotation led to identify nearly 200 putative effectors in H. schachtii. In order to validate the resulted putative effectors, we analysed their expression level (by qPCR) and localization (by in situ hybridization). Sequences that have shown upregulation in post-infective stages and specific localization in esophageal gland were further analysed. Especially, putative effectors that had their functions related to reactive oxidative species, carbohydrate binding, or metabolic processes. We used RNAi to silence our genes of interest and analysed the effect of knocking them down on the level of infection. Interestingly, all genes that were tested have shown a significant effect on parasitism level in one or more of the tested parameters. We expect that our investigation can help understanding more about the processes of cyst nematode parasitism.

Boris SZUREK

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

Tran, T.T[1,#a¶], Pérez-Quintero, A.[1¶], Hutin, H.[1,#b], Wonni, I.[1,#c], Wang, L.[2], Leach, J.E.[3], Verdier, V.[1,3], Cunnac, S.[1], Bogdanove, A.J.[2], Koebnik, R.[1] and Szurek, B.[1,*]

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

Malaika EBERT

Transfer of fungal-derived toxin tolerance to crop plants to engineer resistance to *Cercospora* diseases

Malaika K Ebert[1, 2], Ronnie de Jonge[3,4, 5], Jeffrey C Suttle[2], Jonathan Neubauer[2], Yves Van de Peer[3,4], Bart PHJ Thomma[1], Melvin D Bolton[2]

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[2] *USDA-ARS, Fargo, North Dakota, USA*
[3] *Department of Plant Systems Biology, VIB, Ghent, Belgium*
[4] *Department of Plant Biotechnology and Bioinformatics, Ghent University, Ghent Belgium*
[5] *Department of Plant-Microbe Interactions, Utrecht University, Netherlands*

Fungi of the genus *Cercospora* cause extremely destructive diseases on many crop plants that lead to economic losses worldwide. During infection, many *Cercospora* species produce a light-activated secondary metabolite effector called cercosporin that contributes to fungal virulence. Cercosporin is toxic to many different organisms including plants, bacteria, mice and other fungi with the key exception of *Cercospora* species themselves, which are immune. The metabolic pathway for cercosporin biosynthesis has been well-characterized and was previously thought to consist of eight cercosporin toxin biosynthesis (CTB) genes. Using a phylogenomic approach we discovered that other plant pathogenic fungi outside of the *Cercospora* genus harbor the CTB cluster. Interestingly, based on microsynteny with these genomes, we discovered that the sugarbeet pathogen *C. beticola* has at least five additional genes flanking the established eight-gene cluster that are involved in cercosporin biosynthesis. While three genes are involved in cercosporin production, we discovered two genes that are necessary for auto-resistance to the cercosporin toxin. Mutants that lack both genes were unable to grow on media amended with cercosporin and individual complementation of each of the two genes partially restored auto-resistance. As both genes appear to play a key role in cercosporin auto-resistance, transferring these genes to plants may transfer the resistance mechanism as well. Since cercosporin is a virulence factor and necessary for the fungus to be fully virulent, plants harboring these auto-resistance genes could become insensitive to cercosporin and thereby establish durable resistance to an important group of pathogens.

Fabienne VAILLEAU

How type 3-associated proteins control *Ralstonia solanacearum* species complex pathogenicity?

Fabien Lonjon[1], Marie Turner[1], Céline Henry[2], David Rengel[1], David Lohou[1], Barbara Gomes Ribeiro[1], Claire Péanne[1], Anne-Claire Cazale[1], Nemo Peeters[1], Stéphane Genin[1], Fabienne Vailleau[1, 3]

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

Ralstonia solanacearum species complex (RSSC), causing bacterial wilt disease, exerts its pathogenicity through more than a hundred secreted proteins, many of them depending directly on the functionality of a type 3 secretion system (T3SS). To date, only few type 3 effectors (T3Es) have been identified as individually required for bacterial pathogenicity. In order to identify sets of effectors collectively promoting disease on susceptible hosts, we investigated the role of putative post-translational regulators in the control of type 3 secretion (T3S). We identified three proteins potentially implicated in the control of T3S: the chaperones HpaB, HpaD and the LRR protein HpaG. A shotgun secretome analysis with label-free quantification using tandem mass spectrometry was performed on the GMI1000 strain. 228 proteins were identified, among which a large proportion of T3Es. A focused secretome analysis using the three *hpa* mutants revealed a fine secretion regulation and specific subsets of T3Es with different secretion patterns. We showed that a set of T3Es are secreted in an Hpa-independent manner, whereas others show a positive or a negative Hpa-regulation of their secretion. In parallel, to better understand how these Hpa-proteins control the regulation of the secretion, we looked for Hpa-T3E direct interactions. We screened for HpaB and HpaD interactions with 38 *R. solanacearum* T3Es and we highlighted a set of shared and specific T3Es. In addition, to evaluate the impact of altered T3E secretion on plant pathogenesis, the *hpa* mutants were assayed on several host plants and different host specificities could be observed.

Heike PROCHASKA

Mechanistic aspects of type III-dependent translocation of *Xanthomonas* effector proteins

Heike Prochaska[1] & Ulla Bonas[1]

[1] *Martin Luther University, Biology, Institute of Plant Genetics, Halle, 06120, Germany*

Phytopathogenic bacteria of the genus *Xanthomonas* cause disease in many crop plants thus substantially impacting on agriculture and economy. To successfully infect the host plants pepper and tomato, *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*) translocates effector proteins *via* a conserved type III secretion system directly into plant cells. Here, the type III effectors interfere with plant cellular processes such as immune responses. Selectivity and efficiency of effector translocation is a prerequisite for bacterial multiplication in the plant. MEME-based motif discovery unraveled a conserved sequence in the N-terminal region of more than 20 *Xcv* type III effectors. Extensive mutational analysis confirmed the importance of this motif and influence of secondary structure elements for the efficient translocation of XopB, AvrBs1, AvrBsT and XopQ. Moreover, we succeeded in converting an effector to a non-effector. Lipid-binding assays and localization studies demonstrated that the motif is important for the accumulation of effectors at the bacterial membrane. Thus, our results extend the common view of type III-dependent translocation to a role of protein-lipid interactions.

John Jones

Genomics, effectors and resistance against plant-parasitic nematodes.

Blok, V.C[1]., Danchin, E[2], Espada, M[1,3], Eves-van den Akker, S[4], Gheysen, G[5], Goverse, A[6], Jones, J.T[1], Mantelin, S[1], Mota, M.M[3], Thorpe, P[1] & Varypatakis, K[1]

1 The James Hutton Institute, UK
2 INRA, Sophia-Antipolis, France
3 Evora University, Portugal
4 University of Dundee, UK
5 Ghent University, Belgium
6 Wageningen University, The Netherlands

Genomic and/or transcriptomic analysis has now been used to investigate nematodes from a range of phylogenetic backgrounds and that use diverse strategies to infect plants. Comparative analysis of these data has allowed some of the genomic adaptations that underpin plant-parasitism by nematodes to be determined; these include multiple independent horizontal gene transfer events, evolution of new genes and substantial gene-family expansions. The availability of genome resources has allowed identification of effectors. Candidate effectors can be identified from secreted proteins that are upregulated during parasitism. More recently sequencing of amplified mRNA extracted from the nematode tissues that produce the effectors (the oesophageal gland cells) has provided a more direct route for identification of effectors. In addition, protocols for identifying promotor motifs associated with gland cell expression have been developed and applied in several different nematode species. Given a suitable, and robust, training set of known effector sequences this approach should be more broadly applicable to any plant pathogen for which no amino acid sequence motif that identifies effectors is available. Compared to other plant pathogens, relatively few host targets of nematode effectors have been identified, and this is likely to be a focus for future studies. Analysis of host responses to nematodes have revealed a complex pattern of hormone induction that varies depending on the nature of the infection process of the nematode. It has also become apparent that nematodes use a variety of strategies for suppressing host defence responses. Resistance genes against nematodes have been identified and comparative studies have allowed the function of the recognition process to be probed. Such detailed analyses have already enabled precise modifications to resistance genes that change the recognition spectrum of these genes. Further information on the nematode proteins recognised by resistance genes offers the prospect of engineering durable resistance that recognises previously virulent pathogen strains. In spite of the obvious differences between nematodes and microbial pathogens it has become clear that the general principles of the zig-zag model apply to infection by nematodes. The first nematode PAMP has recently been identified, nematode effectors that suppress host defences have been identified as well as resistance genes and one avirulence gene. New high-throughput sequencing approaches offer the prospect of more rapid identification of nematode resistance genes and the effectors that they recognise and should allow the more rapid development of plants that are resistant against nematodes.

Wenbo Ma

Phytophthora effectors promote infection by suppressing small RNA silencing in plants

Yi Zhai [1,2], Jinqiu He [3], Yingnan Hou [1,2], Duseok Choi [1,2], Wenwu Ye [4], Ariel Kuan [1,2], Jinbiao Ma [3], Wenbo Ma [1,2]

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Phytophthora pathogens cause devastating diseases of crops. Genome sequences of *Phytophthora* species revealed a large number of effector proteins, some of which function inside the host cells to facilitate colonization and infection. Previously, we discovered *Phytophthora* effectors with RNA silencing suppression activity. In particular, *Phytophthora* Suppressor of RNA silencing 2 (PSR2) belongs to a conserved RxLR effector family with tandem repeats of L-W-Y motifs. Using transgenic Arabidopsis plants, PSR2 was found to specifically affect the accumulation of phased small interfering RNAs (phasiRNAs). In this talk, I will report our recent findings on the characterization of PSR2-associating protein(s) in plants, the structure-function analysis of PSR2, and the phasiRNAs affected by PSR2 that contribute to plant defense. I will also discuss our new initiative aiming to identify plant resistance genes targeting PSR2 family effectors.

Session 2

EFFECTORS EVOLUTION AND DIVERSIFICATION (WG3)

Laurent NOËL

Xanthomonas campestris tal effector repertoire evolved from two ancestral genes

Nicolas Denancé[1,*], Boris Szurek[2,*], Erin L. Doyle[3,4], Lisa Fontaine-Bodin[2], Sebastien Carrère[1], Endrick Guy[1], Ahmed Hajri[5], Tristan Boureau[5], Stéphane Poussier[5], Matthieu Arlat[1], Adam Bogdanove[3,4] and Laurent D. Noël[1]

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Transcription Activator-Like (TAL) effectors of *Xanthomonas* are proteins injected into plant cells through the type III secretion system that bind specifically to DNA and activate expression of selected host genes important for plant susceptibility and resistance. Though *tal* genes were absent in the first four genomic sequences available for *Brassicaceae*-infecting *Xanthomonas campestris* (*Xc)*, we show that *tal* genes are broadly distributed in more than half of 49 *Xc* strains isolated worldwide. We used a combination of PCR-based amplification of central repeat regions and SMRT sequencing of seven *Xcc* genomes to yield a near-complete TALome description. Although *Xcc* strains of our collection do not carry more than five *tal* genes, our analysis highlighted an unsuspected diversity of TAL effectors. We identified 53 *tal* genes on *Xc* chromosome or plasmids with 21 distinct DNA recognition specificities from 26 *Xc* strains. *Xc* TALome shows multiple signs of recombination or gene conversion events which probably drove the evolution of *Xc* TALome from at least two ancestral *tal* gene haplotypes. Based on these TAL effector DNA-binding specificities, candidate TAL effector targets were predicted *in silico* in a *Brassica rapa* promoterome. TAL effector-dependent expression could be verified experimentally for eight candidates using qRT-PCR, revealing TAL effector target genes. This large and polymorphic repertoire of TAL effectors opens novel perspectives for the elucidation of TAL-mediated susceptibility of Brassicaceae to black rot disease.

Sebastian EVES-VAN DER AKKER

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

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

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Many effectors in plant-parasitic nematodes are present in families, yet, their evolutionary origins are 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 it appears: Clade 1 are common to nematodes; Clade 2 are common to endoparasites; while the effector genes of Clade 3 are specific to a lineage of plant-parasitic biotrophs. How endogenous enzymes are co-opted as effectors, as demonstrated by effector gene birth in the GS family, involves the gain of the "effector promoter", which we identify, name and validate. Once recruited as an effector, the Clade 3 progenitor underwent massive replication, and the active site residues diverged from that of a canonical GS. GS-like effectors no longer synthesise the same products as endogenous GS *in vitro*. To explore the structural basis for effector diversification, we solved the high-resolution structures of two GS-like effectors, representing the first crystal structures of any kind for a plant-parasitic nematode. We show that the product of this diversification is functional *in planta* as a thiol, thus positioning nematode-derived GS-like effectors in an undescribed synthesis pathway parallel and functionally equivalent to glutathione in the plant redox cycle. Novel biosynthetic pathways unique to a group of plant pathogens presents the possibility of effector-informed drug design against a target specific to these species. Given that GS-like effectors are delivered to, and function within, plant cells, such a drug would be analogous to a medicine for plants, rather than a pesticide.

Krzysztof PAWŁOWSKI

Novel families of pathogen effector kinases in plants. Evolutionary origin and biological function.

Marcin Gradowski[1], Dominika Kamrowska[2], Patrycja Zembek[2], Jacek Hennig[2], Magdalena Krzymowska[2] and Krzysztof Pawłowski[1]

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Recently, several effector kinases have been discovered in pathogenic bacteria. They subvert signalling pathways of their eukaryotic hosts to own advantage. This, and our expertise in discovery of novel kinases [1-3], prompted us to undertake a bioinformatics survey of novel effector kinases in Gammaproteobacteria, a class including many pathogens.Using bioinformatics tools for remote homology detection, we predicted two novel effector families with protein kinase-like fold and likely kinase activity. These two distinct families, HopAG1 and HopBF1, include putative effector kinases from several plant pathogens, e.g. *Pseudomonas syringae*. The latter family is found in several plant but also animal pathogens, from a very diverse set of species. Complex structural domain compositions of some of these novel effectors (e.g. extra protease domains) suggest complex enzymatic mechanisms of action. Also, the kinase-like domains are repeated in a few proteins.Overall sequence comparison and active site analysis of the known and novel effector kinases together with eukaryotic protein kinases suggest different evolutionary origins of effectors, including potential horizontal gene transfer. The discovery of novel putative effector kinase families suggests that the pathogenic effector kinases may be a more common microbial weapon than appreciated to date.Preliminary experimental data is presented that suggests the possible mechanisms of action of the novel effectors and explains the versatility of one of the families, present in pathogens of animals and plants alike.

Lionel GAGNEVIN

Microevolution of *TAL* effector genes in *Xanthomonas citri* pv. *citri* during a Citrus canker epidemic

GAGNEVIN Lionel[1], JAVEGNY Stéphanie[2], BOYER Karine[2], FABRE Sandrine[1], VERNIÈRE Christian[3], PRUVOST Olivier[2]

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The evolutionary potential of *TAL* effector genes is an important parameter in the evolution of pathogens that use those effectors as major virulence factors and for the management of disease resistance. Little is known about the mechanisms and dynamics of the evolution of TAL effectors but their repeated structure and the fact that several paralogs may be present in genomes make them good candidates for efficient intragenic and intergenic recombinations. A population of *Xanthomonas citri* pv. *citri* strains collected in a single field during a Citrus canker epidemic was genotyped using microsatellites. Phylogenetic inferences suggested the epidemic corresponded to a few clonal complexes which may origin from a limited number of migration events. *TAL* effector genes were amplified for 550 strains. Preliminary experiments show that approximately 90% of the strains had the same gene contents and 10% had undergone changes in their TAL effector pattern, losing and gaining one or several bands. These strains usually belonged to specific clonal complexes. A more detailed analysis of the new *TAL* effector paralogs will be essential to decipher the mechanisms of TAL effectors variability and evolution in the field.

Peter THORPE

Genome and RNA sequencing of agriculturally important aphid species reveals conserved and divergent effector sets and host specific effector deployment.

Peter Thorpe [1], Sebastian Eves-Van den Akker[2], Peter Cock[1], Jorunn Bos [1,2]

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Aphids are phloem-feeding insects that cause significant damage to agriculture. Aphids secrete molecules in their saliva which interacts with their hosts at a molecular level, these are termed “effectors”. We used a multi-disciplinary approach of RNA sequencing, saliva proteomics and genome sequencing to investigate the specialist species *Myzus cerasi* and the cereal specialist *Rhopalosiphum padi*. In total, 223 and 421 effectors were predicted for *M. cerasi* and *R. padi* respectively. Predicted *M. cerasi* effectors are significantly further from their nearest neighbours than non-effectors, yet paradoxically, 7.4% of these exist in “effector islands” (17% for *R. padi*). The majority of candidate effectors had sequences no similarity outside aphids, indicating aphid-specific evolution. Moreover, differential expression analysis reveals a two-mode effector usage for *M. cerasi*: effectors for the primary host (Cherry) and a different set for its secondary hosts (Cress and Galium).Our genomic data were clustered with that of previously published aphid species *Acyrthosiphon pisum*, *Diuraphis noxia*, *M. persicae* to identify conserved, species/genus specific and/or novel putative effectors. Many universally conserved effector clusters were identified (205) that contain both previously identified effectors (including *A. pisum* salivary gland sequences), and crucially many novel putative effectors predicted to be involved in the detoxification of plant defence responses. Of the conserved putative effector clusters, ~15% (30) are predicted to be under diversifying selection, including Me10 and C002 containing clusters. Interestingly, scaffolds containing an adjacent pair of universally conserved effectors (Me10 and Mp1) share little to no synteny, indicating high levels of genomic rearrangement.

Johana MISAS VILLAMIL

Host adaptation of the fungal effector Pit2: a case of multiple evolutionary events

Johana Misas-Villamil[1], André N. Mueller [2], Ute Meyer[1], Joji Sernestrand[3], Pitter Huesgen[3], Gunther Doehlemann[1]

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Ustilago maydis is a biotrophic fungus responsible of the corn smut disease in maize. To manipulate its host *U. maydis* secretes a set of effectors into the extracellular interface aiming to downregulate immune responses and so achieving a successful colonization. One of those effectors is Pit2, a secreted cysteine protease inhibitor essential for maintenance of biotrophy (Doehlemann et al., 2011). Pit2 contains a 14 amino acids protease inhibitor domain (PID14) that itself can inhibit cysteine proteases and is essential for fungal virulence (Müller et al., 2013). The closest related barley pathogen *U. hordei* contains a Pit2 ortholog with only low overall sequence similarity to UmPit2 but a conserved PID14 motif. *In vitro* experiments showed that UhPID14 has the potential to inhibit maize cysteine protease activity although its efficiency is reduced compared to UmPID14. Remarkably, *U. maydis* Pit2 deletion mutants complemented with UhPit2 cannot rescue the tumor formation phenotype. To understand this host specificity function of Pit2, its mechanism of action is elucidated in more detail. In maize, UmPit2 is cleaved by apoplastic cysteine proteases releasing PID14 to likely achieve enhanced inhibition of apoplastic cysteine proteases and thus efficiently blocking immune responses. In contrast, UhPit2 is stable in the maize apoplast which might explain the lower suppression of cysteine protease activity *in vivo*. These experiments indicate that Pit2 effector performance is driven by at least two evolutionary steps during host adaptation: 1) Pit2 evolves the capacity to inhibit apoplastic cysteine proteases and 2) Pit2 exploit host proteases to improve their inhibition.

Remco STAM

Using population genomics to understand NLR evolution in wild tomato

Remco Stam[1], Daniela Scheikl[1] and Aurélien Tellier[1]

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NLRs (Nod-like Receptors) are Nucleotide-binding domain and Leucine rich Repeat (NB-LRR)-containing proteins that are important in plant resistance signalling. Many of the known pathogen Resistance (R)-genes in plants belong to the NLRs. Comparative genomics has shown long-term evolutionary relationships of NLRs (e.g. between species) and has shown that different mechanisms might have been in place to accomplish such great NLR diversity as can be observed today. Also in short time scales, functional genes that play such an important role in the plant-pathogen interaction should be under selective pressure, thus studying within and between population differences can help decipher NLR evolution in different habitats and identify possible coevolution with local pathogens. We show the evolutionary patterns of NLR in wild tomato using targeted enrichment sequencing. We have previously shown proof of principle of our methods within one wild tomato population and identified NLR under varying selective pressures. Here we show how we expanded our analysis to multiple populations of the recently sequenced species *Solanum chilense,* a species that shows clear differences in pathogen resistance between populations. We show that NLR evolution does not follow species demography and that there are large variations in selective pressures between populations. Our research forms a starting point for understanding coevolution in wild systems. Additionally, finding NLR under selection will allow identification of the of potentially new and important R-genes or and guide the selection of candidate genes to study the molecular biology of NLR signalling.

Didier THARREAU

What have we learnt on the evolution of avirulence genes? A spotlight on fungal pathogens with particular emphasis on *Magnaporthe oryzae*.

Didier Tharreau[1], Thomas Kroj[2], Elisabeth Fournier[2]

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Map based cloning was successfully used since the early 90s of the last century to identify host specificity and avirulence genes in plant pathogenic fungi. These analyses showed that such genes code mainly for effector proteins. Genome sequencing and comparative genomics accelerated Avr effector cloning and allowed their efficient identification in many major fungal crop pathogens often difficult to treat experimentally as well as the characterization of their distribution and diversification in field populations. Participants of the Sustain Cost Action contributed significantly to this rapid progress in Avr effector identification and on the investigation of their evolution. We will review these recent developments and draw general features, with special emphasis on *Magnaporthe oryzae* were 9 Avr genes have been cloned and studied for their genetic diversity. Based on these published and on additional unpublished results, we identified different evolutionary patterns of Avr effector genes that are probably governed by multiple features such as the genomic environment and the contribution to fungal fitness. Characterizing the structure of Avr effectors also appeared as a major entry point to understand evolution of avirulence genes in M. *oryzae*. From the current state of the art, we will try to speculate on the perspectives in terms of research to understand coevolution between fungal pathogens and their hosts and of applications to manage resistances for increased durability.

Thursday 2nd of March 2017

Session 3

R GENES & HOST TARGETS FOR RESISTANCE BREEDING AND ENGINEERING (WG4)

Stella CESARI

Cytosolic activation of cell death and stem rust resistance by homomeric cereal MLA-family CC-NLR proteins.

Stella Cesari[1], Lachlan Casey[2], Peter Lavrencic[2], Adam Bentham[2], Rohit Mago[1], Chunhong Chen[1], Daryl Webb[3], Sambasivam Periyannan[1], John Moore[1], Maud Bernoux[1], Evans Lagudah[1], Bostjan Kobe[2], Simon Williams[2], Peter Dodds[1].

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Plant proteins of the class of nucleotide-binding oligomerization domain-like receptors (NLRs) are immune sensors which recognize pathogen-derived effectors and induce immune responses. Despite their importance in providing resistance to a wide diversity of plant pathogens, our understanding of the molecular mechanisms that lead to defense signaling is limited. The wheat immune receptors Sr33 and Sr50 belong to the class of coiled-coil (CC) NLRs. They confer resistance against a broad spectrum of field isolates of *Puccinia graminis* f. sp. *tritici*, including the Ug99 lineage, and are homologs of the barley powdery mildew-resistance protein MLA10. Here, we define the minimal CC domains of MLA10, Sr33 and Sr50 that are sufficient for cell death induction and self-association in *Nicotiana benthamiana*. C-terminally truncated CC domains, equivalent in size to an MLA10 fragment for which a homo-dimeric crystal structure was previously determined, fail to induce cell death and do not self-associate *in planta* or *in vitro*. Point mutations in the truncated region also showed the importance of this region for self-association and cell-death, further suggesting that CC self-association is necessary for signaling. Analysis of MLA10, Sr33 and Sr50 CC domains fused to YFP and either nuclear localization or nuclear export signals in *N. benthamiana* showed that cell-death induction occurs in the cytosol. In stable transgenic wheat plants, full-length Sr33 and Sr50 proteins targeted to the cytosol provided rust resistance, whereas nuclear-targeted Sr33 or Sr50 were not functional. These data are consistent with CC-mediated induction of both cell-death signaling and stem rust resistance in the cytosol.

Aranka VAN DER BURGH

SOBIR1-mediated immunity requires conserved tyrosine residues in its kinase domain

Aranka M. van der Burgh[1], Guozhi Bi[1,3], Jelle Postma[2], Silke Robatzek[2] and Matthieu H. A. J. Joosten[1]

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Receptor-like proteins (RLPs) that play a role in resistance of plants to pathogens are transmembrane (TM) proteins that sense non-self molecules and subsequently mediate the activation of downstream signalling leading to plant immunity. Because RLPs have no intracellular kinase domain they constitutively interact with the receptor-like kinase (RLK) SOBIR1 (SUPPRESSOR OF BIR1-1/EVERSHED), providing an intracellular signalling domain to the bi-partite receptor complex. The tomato RLP Cf-4 recognizes the effector Avr4 from Cladosporium fulvum, and SOBIR1 is essential for Cf-4-mediated immunity. SOBIR1 is a positive regulator of immunity, and we show that this RLK induces auto-immunity in N. tabacum and N. benthamiana, which is dependent on the conserved co-receptor BRI1-ASSOCIATED KINASE 1 (BAK1). We show that the SOBIR1 leucine-rich repeat ectodomain, an intact GxxxGxxxG motif in the trans-membrane domain, and a functional kinase domain with some highly conserved tyrosine (Tyr) residues in the kinase domain of SOBIR1 are essential for its auto-immune activity. One Tyr residue, located in the N-lobe, might play a role in the active conformation of the protein, whereas a second Tyr residue, located just after the activation segment, might help to determine the specificity and activity of the kinase. Additionally, we show that these two Tyr residues are critical for SOBIR1 function in Cf-4-mediated HR, supporting our hypothesis that SOBIR1 is involved in signalling downstream of RLPs. Together, these results demonstrate that SOBIR1 auto-immunity and immunity induced in complex with Cf-4 and BAK1 depends on specific regulatory Tyr residues in its kinase domain.

Magdalena KRZYMOWSKA

Regulatory role of the truncated isoform of tobacco N receptor

Marek Żurczak[1], Małgorzata Lichocka[1], Elmon Schmelzer[2], Jane Parker[2], Jacek Hennig[1], Magda Krzymowska[1]

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Resistance to Tobacco Mosaic Virus (TMV) is mediated by the tobacco N protein. Upon recognition of a helicase (p50) domain of the viral replicase in the cytoplasm, the signal is conveyed to nucleus where transcriptional reprogramming occurs. As a result Hypersensitive Response (HR) is activated. Alternative splicing of *N* is crucial for the full TMV resistance. It leads to the production of two transcripts, the full-length *N* and the alternative N_{AL}. The functional relationship between the isoforms of N protein generated *via* alternative splicing remains poorly understood.Our studies show that a truncated protein encoded by N_{AL} (N^{tr}) inhibits HR development triggered upon p50 recognition by the full-length N. This inhibitory effect could be overcome by the forced nuclear localization of N. Consistently, exclusion of N^{tr} from the nucleus, accelerated lesion formations. FRET-FLIM analyses showed that N isoforms formed, in the p50 presence, homo- and hetero-complexes in the cytoplasm. These data suggest that N^{tr} inhibits HR at the phase of oligomerization in the cytoplasm and/or during nucleocytoplasmic transport of N. The fact that SGT1 dependent shuttling of N required N^{tr} supports this model. However the finding that N^{tr} confined to the nucleus abolished HR the most effectively implicates an additional regulatory mechanism executed by N^{tr} in the nucleus. Since N^{tr} bound SPL6 transcription factor, a component required for the N-mediated resistance to TMV, N^{tr} might compete with N for the interaction with SPL6. Collectively, these results suggest that N^{tr} controls multiple steps of N-mediated immune response.

Vivianne VLEESHOWERS

Effector-driven breeding for disease resistance in potato

Vivianne G. A. A. Vleeshouwers, Jaap Wolters, Carolina Galvez-Aguilera, Xiao Lin, Emmanouil Domazakis, Doret Wouters, Richard G. F. Visser

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Potato is the most important non-grain staple crop world-wide and important for food security. However, potato production is threatened by devastating pathogens, such as *Phytophthora infestans* and *Alternaria solani*, causing late blight and early blight, respectively. For achieving a successful infection of their host plants, pathogens employ effectors. To identify novel immune receptors that act upon recognition of such effectors, we are exploiting effectoromics approaches. Effector screens have revealed that wild *Solanum* species recognize multiple types of effectors. Genetic and molecular studies show that immune receptors against diverse effectors has accumulated in these *Solanum* plants during evolution. For breeding for late blight resistance, so far exclusively the nucleotide-binding leucine-rich repeat (NLR) genes have been used. NLR are typically quickly defeated by fast-evolving RXLR effectors, however, *Solanum* plants also contain pathogen recognition receptors (PRR) that recognize conserved effectors. An example is ELR which responds to conserved elicitins of *Phytophthora*. Functional studies with the different immune receptors identified so far show that they can contribute to resistance to *P. infestans*. Recently, we have started searching for early blight resistance and initiated effectoromics for *A. solani*. With the biology of this necrotrophic fungus being completely different from the biotrophic oomycete *P. infestans,* these studies are expected to lead to new insights. Studying the repertoire of immune receptors that recognize the diversity of pathogen effectors will provide profound understanding of the molecular *Solanum* – pathogen interaction. Ultimately, we aim to apply this knowledge for achieving broader and potentially more durable disease resistance.

Erik SLOOTWEG

Inter- and intramolecular interactions regulating the activity of the CNL immune receptors Rx1 and Gpa2 in complex with RanGAP2

Erik J. Slootweg, Rikus Pomp, Jan Roosien, Laurens Voogt, Octavina Sukarta, Aska Goverse

The activities of the coiled coil (CC), nucleotide-binding (NB), leucine-rich repeat (LRR) resistance protein Gpa2 from potato and its close homolog Rx1 are regulated by interdependent interactions of the LRR and CC with the NB domain. Both proteins require a nucleocytoplasmic distribution in the cell for proper functioning. Their CC influences this distribution by association with either nuclear components or with the cytoplasmic co-factor RanGTPase Activating Protein 2 (RanGAP2) which retains the proteins in the cytoplasm. This study is focused on the role of the CC and its interaction with RanGAP2 in the regulation of activation of resistance and cell death responses. Intra- and intermolecular interactions of the R protein have been visualised using Fluorescence Lifetime Imaging Microscopy. Aside from the EDVID motif a few aromatic residues in the N-terminal half of the CC are required for the interaction of the CC with the NB-LRR, but not RanGAP2. Mutations in one region of the CC affect cell death more than resistance signaling, suggesting that the CC plays different roles in regulating either pathway. In addition, mutations in the CC affect the nucleocytoplasmic distribution, underscoring the close link between protein structure, complex formation and subcellular localisation. Disruption of the structure of the CC leads to a more cytoplasmic localisation whereas mutants affected in their interaction with RanGAP2 are no longer sequestered in the cytoplasm by RanGAP2.

Tzion FAHIMA

Evolution and adaptation of wild emmer populations to wheat pathogens

Tzion Fahima[1], Huang Lin[1], Elitsur Yaniv[1,2], Dina Raats[1], Hanan Sela[1,3], Valentina Klymiuk[1], Lihua Feng[1], Assaf Distelfeld[3], Tamar Kis-papo[1], Tamar Krugman[1], Alan H. Schulman[2], Abraham Korol[1]

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Wild emmer wheat, Triticum dicoccoides, the tetraploid progenitor of domesticated wheat, distributed along a wide range of eco-geographical conditions in the Fertile Crescent, has valuable “left behind” adaptive diversity to multiple diseases and environmental stresses. Segregating mapping populations, developed by crossing of selected T. dicoccoides genotypes with T. durum cultivars, revealed numerous loci associated with disease resistance, drought tolerance, high grain protein content, and yield. Furthermore, wild emmer is a promising source of resistance to stripe rust. For example, Yr15 and YrH52 are dominant genes that confer particularly high resistance, while Yr36 confers slow rusting quantitative resistance. Comparative genomics approaches were used to develop high resolution physical maps for Yr15 and YrH52, and for the cloning of Yr36. Yr36 has a unique architecture with a kinase and a START lipid-binding domains, designated WKS hereafter. The distribution and sequence conservation of WKS R-genes were compared with those of NBS-LRR R-genes (e.g. Lr10 and Pm3) among wild emmer natural populations. The sequence diversity of WKS1 was much lower than that of Lr10 and Pm3, indicating that these R-genes, representing different resistance mechanisms, are shaped by different evolutionary processes. Further work is underway to clone Yr15 and YrH52 located on chromosome arm 1BS, using the complete 1BS physical map, constructed by our group, as well as the recently assembled wild emmer

reference genome. These studies demonstrate the potential of wild emmer wheat gene pool for improvement of durum and bread wheats by exploitation of genes that were lost during domestication.

Chih-Hang WU

A complex NLR signaling network mediates immunity to diverse plant pathogens

Chih-Hang Wu[1], Ahmed Abd-El-Haliem[2], Tolga O. Bozkurt[1,3], Khaoula Belhaj[1] Jack H. Vossen[2] and Sophien Kamoun[1]

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Plants rely on nucleotide-binding domain leucine-rich repeat-containing (NLR) proteins to respond to invading pathogens and activate immune responses. An emerging concept in NLR biology is that "sensor" NLR proteins are often paired with "helper" NLR proteins to mediate immune signaling. However, the degree to which NLRs form signaling networks beyond sensor and helper pairs is poorly understood. In this study, we discovered that a large NLR immune signaling network with a complex architecture mediates immunity to oomycetes, bacteria, viruses, nematodes, and insects. Helper NLRs in the NRC (NLR-required fro cell death) family are functionally redundant but display distinct specificities towards diverse sensor NLRs. Several sensor NLRs, including Rx, Bs2 and Sw5b, signal via interchangeable NRC2, NRC3 or NRC4, whereas some other sensor NLRs have a more limited downstream spectrum. For example, Rpi-blb2 signals via NRC4, and Prf signals via interchangeable NRC2 or NRC3 but not NRC4. These helper/sensor NLRs form a unique phylogenetic superclade, with the NRC clade sister to the sensor NLR clades. The network has emerged over 100 million years ago from an NLR pair that diversified into up to one half of the NLRs of asterids. We propose that this NLR network increases evolvability and robustness of immune signalling to counteract rapidly evolving plant pathogens.

Beat KELLER

Host and Pathogen-Informed Strategies to Achieve Durable Resistance in Cereals

Keller, B.

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Due to changing climate, novel host resistance introduced by the breeders and constantly evolving pathogen populations, resistance breeding is a continuous task. The achievement of durable resistance remains the most important, yet difficult goal in resistance breeding. In the last years, there has been an enormous improvement of our molecular understanding of disease resistance in wheat, but also on pathogen evolution. In cereals, and particularly in wheat, the first QTL for quantitative rust resistance have been isolated and reveal novel type of resistance genes, with largely unexplored molecular mechanisms. In addition, work on the improved use of major R genes is developing rapidly based on an increasing number of cloned genes. Strategies for improvement include the targeted mutagenesis of key amino acids in NLR proteins, as well as combination strategies and overexpression. These aspects are currently based on transgenics, but some of the work could eventually also been done using gene editing. Furthermore, the molecular isolation of susceptibility genes proposed to act as toxin receptors has revealed novel possibilities for the management of the necrotrophic Staganospora disease. The novel tools in cereal genetics, including a high quality reference sequence of the wheat genome, will undoubtedly revolutionize gene isolation in wheat. It is foreseeable that all R genes relevant in wheat resistance breeding will be cloned in the next five years. In this research environment, the development of pathogen-informed resistance is promising and highly needed. There has been much progress in the cloning of avirulence

genes from biotrophic wheat and barley pathogens, possibly allowing pathogen-informed strategies for resistance breeding. The translation of all these novel findings into improved resistance in the field remains a main challenge.

Friday 3rd of March 2017

Session 4

PLANT PROTEINS AND PROCESSES TARGETED BY EFFECTORS (WG2)

Mark BANFIELD

Interactions between rice blast effectors and rice HMA domains that underpin disease resistance or susceptibility

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Plant NLRs survey the intracellular environment for the signatures of non-self, typically the presence and/or activity of translocated pathogen effector proteins. Plant NLRs typically contain three domains, CC/TIR, NB-ARC, and LRR, but the prevalence of non-canonical domains in these receptors is being realised. Many of these integrated domains (NLR-IDs) may have their evolutionary origin as virulence-associated effector targets that were recombined into NLRs to act as traps or baits. Little is known about how these NLR-IDs recognise effectors and enable activation of immune signalling. The biochemical and structural basis of recognition of the rice blast pathogen effector protein AVR-Pik with the rice NLR Pikp, via the direct binding of the effector to an heavy metal associated (HMA) domain contained with the NLR, has been described. We have now extended these studies to derive the molecular basis of differential recognition between different AVR-Pik alleles from the pathogen and Pik NLRs from rice. We reveal a correlation between effector binding affinity to the Pik-HMA in vitro and in planta immunity readouts. We continue to explore the potential of engineering NLR-HMAs for bespoke recognition capabilities. Further, we have recently identified the putative AVR-Pik effector virulence-associated targets in rice as small HMA-domain proteins (sHMAs) that may represent the evolutionary ancestors of the NLR-HMA domains. We have shown that AVR-Pik effectors can bind these sHMA proteins in vitro. We are

currently investigating the biochemical properties of these sHMA proteins with the aim of understanding their role in the susceptibility/resistance mechanisms of rice to rice blast.

Frederik BORNKE

Co-option of the host ubiquitin-proteasome system by plant pathogenic bacteria

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Recent evidence suggests that the ubiquitin-proteasome system (UPS) is involved in several aspects of plant immunity and a range of plant and animal pathogens subvert the UPS to enhance their virulence. Proteasome activity is strongly induced during basal defense. We have identified bacterial type-III effector proteins (T3Es) from Xanthomonas campestris pv. vesicatoria (Xcv) and Pseudomonas syringae DC3000 (Pst) that either globally inhibit proteasome activity in their respective host plants to suppress immunity or interfere with the proteasomal turnover of specific regulators of immunity. The Xcv effector protein XopS interacts inside the plant cell nucleus with a protein pair consisting of the transcription factor WRKY40 and an E3-ubiquitin ligase to prevent proteasomal degradation of WRKY40. Stabilization of WRKY40 interferes with the induction of defense genes and attenuates symptom development. A systematic screen for type-III effector proteins from Pseudomonas for their ability to interfere with proteasome activity revealed HopM1, HopAO1, HopG1 and HopA1 as candidates. The mechanistic basis for proteasome inhibition by Pst T3Es is currently unknown. However, we could show that Arabidopsis mutant lines defective in particular subunits of the proteasome support increased bacterial growth of virulent Pseudomonas syringae strains in local leaves. Furthermore, analysis of bacterial growth of Pseudomonas syringae pv. maculicola ES4326 after a secondary infection of systemic leaves revealed that the establishment of systemic-acquired resistance (SAR) is impaired in proteasome mutants, suggesting that the proteasome plays an important role not only the establishment of local immunity but also in defense priming and SAR.

Tolga Osman BOZKURT

RXLR effector PexRD54 employs host components to stimulate biogenesis and relocation of autophagosomes toward haustoria

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A selective form of autophagy organized by autophagy cargo receptors contributes to immunity in plants. Autophagic cargoes are enclosed in autophagosomes, double-membrane vesicles that are marked by ATG8 proteins either to be degraded or relocated. However, we know little about how selective autophagy is regulated and contributes to immunity. To better understand the molecular pathways mediating selective autophagy in plants we exploited *Phytophthora infestans* effector PexRD54 that stimulates autophagosome formation through binding the autophagy protein ATG8CL. We discovered that PexRD54 stimulates autophagosome formation by coupling host vesicle transport regulators to ATG8CL-coated autophagosomes. The host Rab GTPase "Rab8" and a plastid associated UBX domain containing protein interacted with PexRD54 both of which were required for PexRD54 mediated autophagosome formation. Interestingly, live cell imaging revealed that a sub-population of PexRD54 labelled compartments associated to the plastids, indicating that plastids might contribute to PexRD54 triggered autophagy. Finally, PexRD54-labeled autophagosomes are diverted towards haustoria, possibly to allocate cellular resources. Our results implicate effector-mediated employment of host components in autophagosome biogenesis and show that effectors can serve as adaptors targeting protein complexes to co-opt host processes.

Hans THORDAL-CHRISTENSEN

Effort to uncover powdery mildew fungal effectors and their targeting of host processes

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Powdery mildew fungi have intimate interaction with the plant epidermal cells and at the same time they express hundreds of "candidate secreted effector proteins" (CSEPs). These are best studied in cereal powdery mildew fungi attacking wheat and barley. Here genes encoding CSEPs are embedded in transposon-rich domains of the fungal genomes, and evidence suggest that imprecise recombinations have contributed to chromosome dynamics and effector gene amplifications. Gene structure conservation supports that many of the present day CSEP genes are diversified from a common ancestor gene encoding an extracellular RNase. The overall structure of these proteins is often conserved, implying that the ancient RNase provided a useful scaffold for selection of diverse effector protein properties. Transient host-induced gene silencing (HIGS) studies are used to quantify the role of individual effector candidates, and between the research groups in the field, a substantial proportion of the tested CSEPs has been found to

contribute significantly to fungal virulence. Efforts to identify the targeted host process are typically initiated by a yeast 2-hybrid screen, which has allowed finding of a number of defence-related components and mechanisms; some expected - others not. These include pathogenesis-related proteins, heat shock proteins and elements of host cell membrane trafficking.

Anna COLL

Novel crosstalk between ethylene and salicylic acid signalling pathways in virus infected potato unravelled by network analysis

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To understand the mechanisms and dynamics involved in plant-pathogen interaction is crucial for the development of effective crop protection strategies. Here a systems biology approach was adopted to model the complex biological processes associated in the interplay between potato and viral components following potato virus Y (PVY) infection. A previously constructed plant defence signalling model was complemented with information from publically available high-throughput experimental datasets, namely protein-protein interactions, transcription factor regulation and non-coding RNAs. Additionally, datasets describing the interaction between viral and plant components were included. Subsequently, the network was transferred from model plant Arabidopsis to potato using published orthologue information. The constructed large knowledge network was superimposed with co-expresssion data from two time-series transcriptome datasets of sensitive, tolerant and hypersensitive resistant responses of potato to PVY. Network analysis of the generated networks offers new insights into the plant-pathogen interaction by expanding the knowledge on critical components of plant defence signalling. One of the most interesting findings, which was also functionally validated in potato, is the previously unknown crosstalk between the ET and SA signalling pathways.

Sebastian BECKER

Flexibility in TALEs – an adaption to variable targets and plant resistance

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Plant pathogenic bacteria of the genus *Xanthomonas* specifically induce the transcription of target genes by translocating transcription activator-like effectors (TALEs) into host cells. TALEs contain highly conserved 34aa-repeats for the binding of DNA in a simple one repeat to one nucleotide manner. Rare repeat-variants harbor small deletions or insertions, resulting in repeats that differ from the usual repeat consensus. Those so called aberrant repeats enable the TALE to conditionally recognize target sequences with a -1 nucleotide frameshift. Likely, this mechanism is based on the aberrant repeat looping out upon encountering a sequence with a frameshift, whereas the repeats can perfectly align into the repeat array at sequences without the frameshift.
We analyzed these natural occurring repeat variants in further detail, showing the impact and limits of multiple aberrant repeats in a single TALE. Those experiments demonstrated that it is possible for a TALE not only to tolerate multiple aberrant repeats but to bind sequences with larger deletions by simply looping out several repeats simultaneously. Furthermore, we will present three new repeat variants not reported before and show that not all natural occurring aberrant repeats allow for frameshift binding. We pinpointed this difference to the position and nature of altered amino acids. We also analyzed the first known natural TALE harboring two aberrant repeats and identified its target to be the well-known virulence target *OsSWEET13* from rice. As an adaptive mechanism in the molecular arms race between pathogen and host, aberrant repeats can help TALEs to overcome INDEL-based resistance mechanisms.

Georgy POPOV

The *Xanthomonas* type III effector XopAE encodes an E3 ubiquitin ligase that inhibits PAMP-triggered immunity

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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. Collectively, T3S effectors suppress plant PAMP-triggered immunity (PTI), alter metabolism and gene expression of the host for the benefit of the pathogen. The *XCV0408Ψ* gene, whose homologues in other *Xanthomonas* strains encode the XopAE effector, was annotated as a pseudogene in the *Xe* 85-10 genome because of a frameshift mutation. By expression and translocation analysis of *XCV0408Ψ* locus, we identified an operon with two open reading frames encoding for HpaF and truncated but functional T3S effector XopAE. The truncated XopAE translocation was mediated by HpaF. XopAE displayed the ability to interfere with PTI as it inhibited the activation of a flg22-responsive promoter and prevented callose deposition at the plant cell wall. PTI signaling involves activation of mitogen-activated protein (MAP) kinase cascades. However, expression of XopAE did not affect flg22-mediated phosphorylation of the MPK3 and MPK6 MAP kinases indicating that this effector interferes with PTI signaling downstream to immunity-associated MAP kinase cascades. Homology modeling of the XopAE C-terminus revealed a XL-box fold of an E3 ubiquitin ligase domain. Follow up *in vitro* biochemical analysis showed E3 ligase activity and protoplast assay demonstrated that XopAE suppress PTI by exploiting the ubiquitin system of the host. Together our findings provide new insights into the function, biochemical properties and evolution of a previously uncharacterized *Xe* effector protein.

Nemo PEETERS

A selection of my favourite effector-stories from the last years

I would like to take the opportunity of this last COST-SUSTAIN meeting to give my own personal top list of cool effectors and show how they interfere with the host plant for the benefit of the pathogen. Colleagues from this COST action have dedicated a lot of time to study these effectors, I would like to capture their essence in a series of snapshots. My objective is to share with you how inspiring these stories can be.

POSTERS

WG	N°	P°	First Name	Name	Abstracts
1	1	42	Sabina	BERNE	Studies of virulence factors in *Verticillium nonalfalfae*
1	2	43	Martin	DARINO	Study of *Ustilago maydis* effectors targeting plant auxin signaling
1	3	43	Barbara Sasa	GERIC STARE SIRCA	Structure and presence of map-1 genes, putative effectors in tropical root-knot nematodes *Meloidogyne ethiopica* and M. *luci*
1	4	44	Ece börteçine	KASAPOGLU	Identification and Comparative Transcriptomic analysise of *H.schachtii, H. avenae*, and *Globodera pallida*
1	5	44	Nada	KRAŠEVEC	Diversification of *actinoporin*-like proteins
1	6	45	Parvathy	KRISHNAN	Role of a cellulase in the virulence of the wheat Pathogen *Zymoseptoria tritici*
1	7	46	Jinling	LI	Identification and characterization of the *Verticillium dahliae* effector that is responsible for cotton defoliation
1	8	46	Catherine	LILLEY	C-terminally encoded plant peptide (CEP) hormone domains from the plant parasitic nematode *Rotylenchulus reniformis* have dual roles *in planta*
1	9	47	Matias	PASQUALI	A transposon-tagging approach identifies a leotiomycete-conserved gene involved in the regulation of pathogenicity, fungicide sensitivity, mycotoxin production as well as fitness in F. culmorum and F. graminearum
1	10	48	Carsen	PEDERSEN	Did *Blumeria graminis* effector gene promoters evolved to respond to an alkalinizing environment?
1	11	48	Helen	PENNINGTON	Genetic basis of translocation *of Magnaporthe oryzae* avr-pik into rice cells
1	12	49	Marc	PLANAS	Protease activities induced in the apoplast of a resistant tomato cultivar in response to *Ralstonia solanacearum* infection
1	13	49	Diego	RUBIALES	Identification of necrotrophic effectors produced by *Didymella pinodes* and *Botrytis fabae* and their use in legume breeding for disease resistance
1	14	50	Sebastian	SCHORNACK	A mycorrhiza fungus effector promotes symbiosis and infection by an oomycete pathogen
1	15	51	Natasa	STAJNER	Prediction of novel microRNAs in soil borne plant pathogen Vertcillium nonalfalfae using NGS approach
1	16	51	Marc	VALLS	Ralstonia solanacearum effectors expressed during potato infection
1	17	52	Pieter. J	WOLTERS	Studying effectors from A. *Solani* and early blight resistance in potato
1	18	53	Jinbin	WU	A Pseudomonas syringae pv. tomato (Pst) DC3000 type three secretion system (TTSS) effector targets and interrupts the SOBIR1-mediated signalling pathway
2	1	54	Ahmed	ABD-EL-HALIEM	Plant-Thrips arms race: Identification of Thrips Effector Proteins and their *in planta* Targets
2	2	55	Nisha	AGRAWAL	The *Sporisorium reilianum* effector Sad1 targets the maize RGLG2-like protein to suppress apical dominance

					in maize ears
2	3	56	Philip	ALBERS	The immune kinase PBS1 phosphorylates a remorin protein which is also targeted by the bacterial effector HopZ1a
2	4	56	Maria	ERCOLANO	Integrate –omics approaches for undercovering tomato tuta absoluta interaction
2	5	57	Johannes	FAHRENTRAPP	Gene expression in tomato leaves during pathogen infection
2	6	58	Jernej	JAKSE	In-silico prediction and validation of potential gene targets for viroid derived small RNAs in hop plants
2	7	59	Marek	KOTER	The cornucopia of isomiRs tunes host response to *Globodera rostochiensis* parasitism
2	8	59	Tjasa	LUKAN	Promoter analysis of several genes involved in plant immune response
2	9	60	Mateusz	MATUSZKIEWICZ	Complex PCD regulation during compatible plant-nematode interaction
2	10	61	Sergio	MAURO	Proteomic study of SUMOylation during *Solanum tuberosum* / *Phytophthora infestans* in (in-) compatible interactions
2	11	61	Oliver	NAGEL	Impact of *Xanthomonas* type III-effector proteins on the transcriptome of tomato
2	12	62	Cyrus Raja Rubenstein	SABBAGH	Towards a global interactomic map between the *Ralstonia solanacearum* species complex core type 3 effectors and the tomato proteome
2	13	62	Heike	SEYBOLD	Actio est Reaction Successful bypassing of wheat immune responses by a fungal pathogen
2	14	63	Suayib	USTUN	How plant pathogenic bacteria co-opt the proteolytic degradation pathways
2	15	63	Patrycja	ZEMBEK	The function of the N-terminus of HopQ1 effector from *Pseudomonas syringae*
3	1	64	Norfarhan	BINTI MOHD ASSAAD	Genome-wide association mapping identifies multi-locus resistance evolution to fungicides in the barley pathogen *Rhynchosporium* commune
3	2	65	Beatrice	CORSI	Using Multiparent Advanced Generation Inter-Cross (MAGIC) populations to investigate wheat leaf spot group (LSG) host–pathogen interactions in the UK
3	3	66	Marta	GRECH-BARAN	Natural variation of HopQ1 TTSS effector from *Pseudomonas syringae*
3	4	67	Yogesh	GUPTA	Field dynamics and patho-genomics of Asian soybean rust pathogen *Phakopsora pachyrhizi*
3	5	68	Tina	JORDAN	The TAL effectors avrBs3 and avrBs4 are widespread in *Xanthomonas euvesicatoria* field strains, but show limited diversity
3	6	68	Pezhman	SAFDARI	A genomic approach to unravel plant-pathogen coevolution in the wild
3	7	69	Jadwiga	SLIWKA	Sequence diversity and expression of AvrSmira1 and Avr-vnt1 effectors of *Phytophthora infestans* in virulent and avirulent isolates

3	8	70	Cécile	THOMAS	Life-history traits of Phytophthora infestans condition the effectiveness of resistance induced by PAMPs in potato
3	9	71	Maria Carlota	VAZ PATTO	Portuguese common bean landraces vs. Fusarium *oxysporum:* a diverse array of resistances
3	10	72	Ronja	WONNEBERGER	Identification of resistance to *Drechslera teres* in Norwegian barley and population structure of a Norwegian D. teres population
4	1	73	Carolina	AGUILERA-GALVEZ	Recognition specificity of AVR2 effectors from P. infestans in Solanum native of Mexico and Peru
4	2	74	Balazs	BARNA	Some aspects of host and nonhost interactions of powdery mildews
4	3	75	Aleksandra	BIALAS	Evolution and specialization of an NLR-integrated domain
4	4	76	James	CROCKRAM	An Effector- and Genomics-Assisted Pipeline for Necrotrophic Pathogen Resistance Breeding in Wheat
4	5	77	Gulay	DAGDAS	Hotspots of NLR receptor diversification and evolution of NLR-kinases in wheat
4	6	78	Rowena	DOWNIE	Mapping the wheat *Snn3-B1* locus conferring sensitivity to the *Parastagonospora nodorum* necrotrophic effector SnTox3 using an eight founder multi-parent advanced generation intercross (MAGIC) population and the Avalon x Cadenza doubled haploid population.
4	7	78	Zane	DUXBURY	Understanding and engineering immune receptor complexes in plants
4	8	79	Valerie	GEFFROY	DNA methylation of NB-LRR sequences in common bean genome
4	9	80	Anne	GIESBERS	Effector-mediated downy mildew resistance discovery in nonhost lettuce accessions
4	10	80	Amelie	HECKMANN	Integration of protein kinase in plant NLR receptors: a new way to recognise pathogens
4	11	81	Matthieu	JOOSTEN	SOBIR1 plays a central role in signaling by receptor-like proteins
4	12	82	Sung-yong	KIM	Editing a net blotch susceptibility gene for disease resistance in barley using CRISPR
4	13	82	Valentina	KLYMIUK	Towards positional cloning of a new stripe rust resistance gene YrG303 derived from wild emmer wheat
4	14	83	Marjin	KNIP	Studying the link between DNA-damage and NLR-mediated immune responses
4	15	84	Marc-Henri	LEBRUN	Wheat Effector Assisted Breeding for Resistance to Fungal Pathogens (WEAB)
4	16	85	Min	LIN	Can sensitivity to necrotrophic effectors explain differences in host resistance to *Parastagonospora nodorum* blotch in European winter wheat?
4	17	85	Charlotte	NELLIST	Improving disease resistance in strawberry
4	18	86	Liliya	PYLYPENKO	Identification of sources of resistance to cereal cyst nematode in common wheat cultivars from Ukraine
4	19	86	Yuan	QIN	The type-III secretion system (T3SS) of *Pseudomonas syringae pv. syringae* 61 (Psy61) may be recognized in *Triticum aestivum*

4	20	87	Manon	RICHARD	Deciphering the role of NLR immune receptors DNA binding / damage in plant immunity
4	21	87	Anja	RUUD	SnTox3-Snn3 as a major determinant of field susceptibility to *Septoria nodorum* leaf blotch in the SHA3/CBRD × Naxos population
4	22	88	Octavina	SUKARTA	Exploring the *Resistosome* of the Potato CC-NB-LRR Immune Receptor Rx1
4	23	88	Michel Vanessa	VAN THOURNOUT HOSTYN	The impact of different sequencing technologies on R-gene cloning strategies
4	24	89	Andrea Paola	ZULUAGA	Studying the BED protein domain as a new player in plant tolerance to biotic and abiotic stresses

ABSTRACTS OF POSTER PRESENTATIONS

Working Group 1

Pathogen effectors and virulence

1 – 1 Sabina BERNE

Studies of virulence factors in *Verticillium nonalfalfae*

Marko Flajsman[1], Jernej Jakse[1], Sebastjan Radišek[2], Nataša Stajner[1], Kristina Marton[1], Helena Volk[1], Sabina Berne[1], Branka Javornik[1]

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Verticillium wilt of hops has emerged as a serious threat to hop production in Europe due to outbreaks of highly aggressive lethal strains of *Verticillium nonalfalfae*. In this contribution we will present our recent identification of several candidate secreted effector proteins (CSEP) in fungus identified by various approaches (gene expression analysis of host-pathogen interactions, comparison of the genomes of strains with different virulence and by bioinformatics of the available secretome). Deletion mutants of these CSEP showed reduced, increased and unchanged virulence in the pathogenicity assay using hop (*Humulus lupulus* L.), a primary host. The main criteria for selection of candidate genes was their *in planta* expression after plant inoculation with the fungus, as identified by proteomic and transcriptomic host-pathogen interaction studies, high expression of the genes derived from the lethal specific genomic region of the aggressive pathotype during their growth in xylem simulating media and bioinformatically selected CSEP from the gene models of the *V. nonalfalfae* secretome. Altogether twelve out of twenty-four so far identified CSEP were successfully tested in the pathogenicity test and those implicated in virulence are being further characterized by localization, infiltration and interaction studies to unravel the function of selected candidates in *V. nonalfalfae* virulence.

1 – 2 Martin DARINO

Study of *Ustilago maydis* effectors targeting plant auxin signalling

Martin Alejandro Darino[1], Janos Bindics[1], Armin Djamei[1]

[1] *Gregor Mendel Institute of Molecular Plant Biology, Vienna, Austria.*

The smut fungus *Ustilago maydis* causes gall formation on all aerial parts of its host plant maize. The pathogen secretes numerous manipulative molecules, so called effectors responsible for the suppression of host defense responses and the dramatic morphological changes on the host side. Despite their functional importance during biotrophy, most putative effectors encode for proteins of unknown function. In a heterologous screen performed in *Nicotiana benthamiana*, several putative *U. maydis* effectors have been identified which have in planta auxin signaling-inducing capacity and they were designated as inducer of auxin signaling (IAS). As auxin is one of the central growth hormones in plants, the identified putative fungal effectors could be involved in the gall inducing capacity of *U. maydis* and/or suppression of the host defense. The functional characterization in detail of the identified *ias* genes by studying: their role during biotrophy, their localization as well as their host sided interaction partner will provide new insights on how this fungal pathogen manipulates its host. Furthermore, the identification of the IAS host sided interaction partners might reveal yet unknown proteins linking auxin signalling with immunity and might reveal general host sided hubs that are also targeted by other biotrophic pathogens.

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1 – 3 Barbara GERIC STARE

Structure and presence of map-1 genes, putative effectors in tropical root-knot nematodes *Meloidogyne ethiopica* and *M. luci*

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Root-knot nematodes (RKN) of the tropical group including *Meloidogyne ethiopica* and *M. luci* are highly polyphagus and damaging pests causing great loses in crop production. *Meloidogyne ethiopica* has been detected and reported for several times in Europe (Slovenia, Italy, Greece, Turkey and Portugal). However, a new sister species *M. luci* was described recently which classifies all reported populations of *M. ethiopica* in Europe as *M. luci* with esterase isozyme pattern as the most distinguishing character between the two otherwise very similar species. The finding of virulent populations that can reproduce on tomato plants bearing *Mi*-1 resistance gene adds to the concern on damaging potential of this pest. A set of taxonomically restricted genes found only in tropical RKN, *map*-1 gene family encodes expansin-like proteins that are putative effectors. The loss of one copy of *map*-1 gene in *M. incognita* near-isogenic lines has been reported to correlate with the break of *Mi*-1 mediated resistance in tomatoes. In order to test if the same can be observed in *M. luci*, we have determined the sequence of repetitive domains in *map*-1 genes in a set of *M. ethiopica* and *M. luci* populations, including avirulent and virulent *M. luci* population from Turkey. The presence of two *map*-1 genes was determined in all of our tested populations; designated as *map*-1.1 and *map*-1.2 with the same structure of repetitive domains. Both genes were present in avirulent populations as well as in the virulent population. The emergence of virulence does not correlate with the loss of *map*-1 genes in *M. luci*.

Acknowledgements: supported by ARRS (P4-0072, BI-TR/15-17-008) and TUBİTAK (214 O 667).

1 – 4 Ece Börteçine KASAPOGLU

Identification and Comparative Transcriptomic analysis of *H.schachtii, H. avenae* and *Globodera pallida*

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Sedentary plant-parasitic cyst nematodes cause significant losses in various crops. As a basis of parasitism, these nematodes select a single root cell, activate its cell cycle and thus induce the formation of a multicellular nurse cell system. In this study, we identify common and species specific-putative secretory proteins among the cyst nematode species *H. schachtii*, *H. avenae*, and *G. pallida*, by comparing their transcriptomes and secretomes. Among the common putative secretory protein within the 3 tested nematode species, we identified a bactericidal permeability-increasing and lipopolysaccharide-binding protein (BPI-LBP). These sequences have a high homology with BPI (Ce-BPI) of *Caenorhabditis elegans. Heterodera schachtii* BPI (Hs-BPI) was found to be expressed within the esophageal gland. Knocking down Hs-BPI by dsRNA has affected negatively the parasitism of *H. schachtii*. Based on our results we suggest that Hs-BPI plays a role in *H. schachtii* parasitism.

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1 – 5 Nada KRAŠEVEC

Diversification of *actinoporin*-like proteins

Nada Kraševec[1], Sabina Belc[1,2], Maruša Novak[2], Kristina Sepčić[2], Peter Maček[2], Gregor Anderluh[1]

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Actinoporins are potent cytolytic toxins isolated from sea anemones. Superfamily of actinoporin-like proteins (ALP) comprises diverse protein families sharing structural similarity (a rigid beta-sandwich flanked by two alfa-helices) but low sequences similarity. We performed genome mining for ALP in fungal kingdom and comparison of lifestyles, analysis of genome loci, promoters, transcription patterns, secretion and protein signatures, literature search for described function and application. When mining fungal genome data several challenges limit comparisons (Meyer *et al.*, 2016): The quality of genome sequences; The accuracy of gene calling; Versions of sequences or annotation for the same genome; A single genome sequence available (a lab pet); History of species names; Substantial variation in experimental conditions; Omics data often usable for researchers with bioinformatics skills only. Genome datasets hosted at different resources were analysed by some useful tools, each offering benefits and limitations: NCBI, Mycocosm, AspGD, E-Fungi, PFAM, PHYRE, CLUSTALW and SecretomeP. A pool of ALP was collected out of ambiguous number of screened species. We determined distribution for fungal fruit body lectins, necrosis inducing proteins and aegerolysins. The occurrence was dispersed; aegerolysins and NPP1 were overrepresented, while FB lectins were rare. No obvious correlation to taxonomy or lifestyle was observed although fungal lifestyle is difficult to describe univocally. ALP can be considered as noncore proteins and a part of them as small secreted proteins, often without recognizable signal peptide. Some aegerolysins co-distributed with MACPF/CDC. Some potential biotechnological applications of aegerolysins are already evident, despite the limited knowledge at present (Novak *et al*., 2015).

1 – 6 Parvathy KRISHNAN

Variable levels of melanization in *Zymoseptoria tritici* is mediated by differential expression of a transcription factor ZMR1

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In fungal pathogens melanin plays a major role in tolerating harmful radiation, virulence, and resistance to fungicide. Even though the biosynthetic pathway of melanin is highly conserved across different fungal species, we observed a high variability in the degree of melanization among the different isolates of the wheat pathogen *Zymoseptoria tritici*. A mapping population derived from a cross between two Swiss isolates 3D1 (lighter) and 3D7 (darker) was used to understand the genetic basis of the differences in melanin accumulation. Of the 6 candidate genes identified on chromosome 11, two belong to the melanin biosynthetic gene cluster, including a homolog of a transcription factor *Cmr1* (Colletotrichum Melanin Regulation). In other fungal species CMR1 is known to regulate the expression of the genes in the melanin biosynthetic gene cluster. Disruption of *Cmr1* homolog in *Z. tritici* leads to loss of melanin deposition, confirming its role in melanin biosynthesis. We did not observe any sequence polymorphism in the coding sequence of CMR1 that could explain the differences in melanin accumulation between 3D1 and 3D7. However *Cmr1* expression levels were significantly lower in the lighter isolate (3D1) compared to the darker one. Interestingly, we observed insertions rich in transposable elements 1.8 kb upstream of the start codon of *Cmr1* only in 3D1 and not in 3D7. Preliminary data indicates that *Cmr1* expression in 3D1 is repressed by the transposable element. Thus, gene expression level polymorphism of a major melanin regulator, leads to differences in melanin accumulation in *Z. tritici* and is linked to the presence /absence of a TE that regulates its expression.

1 – 7 Jinling LI

Identification and characterization of the *Verticillium dahliae* effector that is responsible for cotton defoliation

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Plant pathogens from diverse taxonomic origins have been shown to secrete effector proteins into host plants to manipulate host physiology and establish infection. Verticillium dahliae is an asexual soil-born, fungus that causes Verticillium wilt disease in a wide range of crops, including cotton and olive. V. dahliae strains have previously been characterized as defoliating and non-defoliating strains based on their ability to cause defoliation on cotton, but the V. dahliae gene(s) that are involved in cotton defoliation remain unknown thus far. Here, we present a comparative genomics study defoliating and non-defoliating strains of V. dahliae that enabled us to identify a region of about 20 Kb that specifically occurs in defoliating strains. In this region, we were subsequently able to uncover a single highly-expressed gene that encodes a putative effector protein. Currently, we are performing experiments to confirm the role of this effector in cotton defoliation.

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1 – 8 Catherine LILLEY

C-terminally encoded plant peptide (CEP) hormone domains from the plant parasitic nematode *Rotylenchulus reniformis* have dual roles *in planta*

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The interaction between sedentary plant parasitic nematodes and their host is mediated by nematode effectors. These secreted proteins are responsible for the induction and maintenance of the nematode feeding site within the host root. We have discovered a large and diverse family of effector genes, encoding C-terminally Encoded Peptide (CEP) plant hormone mimics (RrCEPs), in the syncytium-forming, reniform nematode Rotylenchulus reniformis. Unlike CEP genes from all other organisms, whether plant or nematode, those cloned from R. reniformis contain one intron per domain sequence, regardless of the number of tandem domains that are present. Together with the distant phylogenetic relationship of R. reniformis to the only other CEP-encoding nematode genus identified to date (Meloidogyne), this suggests CEPs likely evolved de novo in R. reniformis. We have characterised the first member of this large gene family (RrCEP1), demonstrating its expression in the effector-producing pharyngeal gland cell and significant up-regulation during the biotrophic phase of the life cycle. RrCEP1 encodes a functional CEP domain which significantly up-regulates expression of an Arabidopsis nitrate transporter, while simultaneously reducing primary root elongation. The same CEP domain also limits syncytium expansion in Arabidopsis for the non-CEP containing cyst nematode Heterodera schachtii. CEP effectors of R. reniformis may therefore represent a two-fold adaptation to sustained biotrophy by increasing host nitrate uptake whilst limiting the size of the syncytial feeding site produced.

1 – 9 Matias PASQUALI

A transposon-tagging approach identifies a leotiomycete-conserved gene involved in the regulation of pathogenicity, fungicide sensitivity, mycotoxin production as well as fitness in *F. culmorum* and *F. graminearum*

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A double-component system based on the ability of the *impala* transposase to heterologously activate the miniature inverted-repeat transposable element *mimp1* of *Fusarium oxysporum* to generate a library of transposon-tagged genes in *F. culmorum* was developed. By screening the library, an insertion mutant showed reduced pathogenicity and increased fungicide sensitivity. The mutant was characterised by identifying the insertion using splinkerette PCR and the corresponding gene was isolated. KO mutants were generated in both *F. graminearum* and *F. culmorum.* The overall gene expression profile in *F. graminearum* was determined. A phenotypic array for both mutants and wildtypes in the 2 species was performed and analysed. The KO strains lost virulence, decreased significantly DON production in vitro and *in planta* (crown rot) and showed higher sensitivity to azoles. We describe here the hypothetical role of the gene based on its protein structure and conservation in the fungal kingdom and suggest the importance of this protein in the fitness of the pathogen, making it an interesting target for new fungicides.

1 – 10 Carsen PEDERSEN

Did *Blumeria graminis* effector gene promoters evolved to respond to an alkalinizing environment?

Carsten Pedersen[1] *and Hans Thordal-Christensen*[1]

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Plant pathogenic fungi undergo a transcriptional re-programing when they infect their hosts, and many studies have shown that effector-encoding genes are among the highest expressed genes in fungal structures, such as haustoria or feeding hyphae inside the plant tissue, while they are almost absent in epiphytic fungal tissue. The genome of the barley powdery mildew fungus, Blumeria graminis f.sp. hordei, encodes about 500 candidates for secreted effector proteins (CSEPs) of which many are highly induced and highly expressed in haustoria. We searched the CSEP promoters for cis-elements that could explain their dramatic up-regulation in planta and found a few candidates for such elements. One of these, GCCAAG, is a well-known alkaline-pH response element recognized by the transcription factor PacC that activate transcription of alkaline-expressed genes and has been found to be an important virulence factor in several pathogenic fungi. Plant infections caused by fungi are often associated with an increase in the pH of the host tissue and recently it has been found that some fungi even secretes plant alkalinizing peptides to increase infection (Masachis et al. 2016, Nature Microbiology 1, 16043). Therefore, we speculate that effector genes are "hitchhiking" on this mechanism. This idea implies that the GCCAAG-element has evolved in effector gene promotors to induce high transcription when the fungus is in an alkalinizing plant environment.

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1 – 11 Helen PENNINGTON

Genetic basis of translocation of *Magnaporthe oryzae* avr-pik into rice cells

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Fungi and other filamentous pathogens secrete effectors inside host cells to help establish a successful infection in the host plant. However, the mechanism by which effectors translocate into the plant is unknown (Petre and Kamoun, 2014, PLOS Biology, 12:e1001801). The effector AVR-Pik is produced by the rice blast pathogen Magnaporthe oryzae and binds to the rice intracellular NLR immune receptor Pikp-1, which subsequently activates Pikp-2. The allele AVR-PikD binds the HMA domain of the Pikp-1 protein with nanomolar affinity, indicating that AVR-Pik is translocated inside the host cell. In addition, the crystal structure of the AVR-PikD/Pikp-HMA complex has been resolved (Maqbool et al. 2015, eLife, 4:e08709) revealing the contact points between the effector and the NLR protein. We have performed an alanine mutant scan for single amino acid mutants of AVR-PikD, and investigated the activity of the mutants by performing a Hypersensitive Response (HR) screen in Nicotiana benthamiana. Work is ongoing to further characterise the active mutants, and to determine which are translocated from M. oryzae into Pikp rice. The objective of this project is to take advantage of this basic knowledge to identify AVR-PikD residues that are required for translocation inside rice cells but do not affect detection by Pikp-1. This will help us to determine the genetic basis of translocation of AVR-Pik into rice cells.

1 – 12 Marc PLANAS

Protease activities induced in the apoplast of a resistant tomato cultivar in response to *Ralstonia solanacearum* infection

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Ralstonia solanacearum is a soil-borne pathogen that causes the widespread devastating disease known as bacterial wilt. The most effective way to fight the disease seems to be the use of resistant cultivars, but little is known about the molecular mechanisms conferring resistance to R. solanacearum. The first interaction between R. solanacearum and its hosts takes place in the apoplast, but the changes in protease activities upon infection remain mostly unexplored. In this work, we characterized using activity-based protein profiling coupled with mass spectrometry the apoplastic protein activity of a susceptible (Marmande) and a resistant (Hawaii7996) tomato variety when challenged with R. solanacearum. A quantitative analysis of the identified proteins indicate that tomato papain-like cysteine proteases (PLCPs) and serine hydrolases (SHs) were induced in the presence of R. solanacearum and, in some cases, the intensity of these responses was enhanced in the resistant cultivar. Among them, we found that three SHs from the P69 family of subtilases (P69B, P69C and P69F) and two PLCPs (Rcr3 and Pip1) were important for the response against R. solanacearum. Besides that, we will also present proteomic data regarding the whole apoplastic proteomes of infected and uninfected tomato, comparing both susceptible and resistant cultivars

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1 – 13 Diego RUBIALES

Identification of necrotrophic effectors produced by *Didymella pinodes* and *Botrytis fabae* and their use in legume breeding for disease resistance

Diego Rubiales[1], Alessio Cimmino[2], Marco Masi[2], Antonio Evidente[2], Sara Fondevilla[1] and Angel M. Villegas-Fernadez[1]

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Ascochyta and *Botrytis* species cause major diseases on legume crops. Fungi from both genera are well known phytotoxins producers. Based on collaboration between CSIC and Univ. Naples necrotrophic effectors produced by *Didymella pinodes* and *Botrytis fabae* have been identified and characterized chemically and biologically. Their use in resistance screenings are being tested and will be critically discussed.

1 – 14 Sebastian SCHORNACK

A mycorrhiza fungus effector promotes symbiosis and infection by an oomycete pathogen

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The majority of all land plants benefit from a symbiosis with arbuscular mycorrhiza fungi (AM fungi). The AM fungus *Rhizophagus irregularis* can colonise roots of *Medicago truncatula*, barley, and *Nicotiana benthamiana*. Recent work has shown that similar to pathogens symbiotic fungi utilise effector proteins to suppress immunity and support colonisation. Compared to the knowledge in pathogens, not much is known about effectors of AM fungi and the root processes they target. We hypothesized that both, beneficial and pathogenic filamentous microbes may rely on similar host processes to support their colonisation. We thus surveyed 24 *R. irregularis* genes (*R1-R24*) encoding secreted proteins. Nine genes are expressed during *M. truncatula* colonisation but 22 genes are expressed during *N. benthamiana* colonisation. *R5* promotes mycorrhiza symbiosis but also infection by the unrelated filamentous root pathogen *Phytophthora palmivora* when expressed in *N. benthamiana* roots. This suggests that R5 targets a root process relevant for diverse filamentous microbes. In support, R5-homologous genes can be found in other symbiotic fungi but also in root pathogens. Furthermore, R5 can suppress disease resistance gene-mediated responses and we are currently studying whether it acts at the stage of effector perception or in downstream signalling. Through interaction studies we have identified candidate root-expressed plant targets. I will present progress in identifying the host plant process underlying colonisation of unrelated symbiotic and pathogenic filamentous microbes. This may inform about symbiosis-supporting resistance breeding.

1 – 15 Natasa STAJNER

Prediction of novel microRNAs in soil borne plant pathogen *Vertcillium nonalfalfae* using NGS approach

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RNA interference is an evolutionary conserved eukaryotic mechanism of post transcriptional regulation of gene expression mediated by various classes of small RNAs (sRNAs). Micro RNA-like sRNAs (milRNAs) have been recently shown to exist in filamentous fungi. Moreover some of the plant pathogens use these classes of sRNAs during the infection process like effector molecules. Therefore it is necessary to study sRNAs in fungal plant pathogens. To date no milRNAs have been reported in the phytopathogenic fungus *Verticillium nonalfalfae* (Vna), a soil borne plant pathogen causing vascular wilt in many important crops worldwide. Two pathotypes of Vna are threatening hop production, with lethal strain causing severe symptoms and complete dieback of the plant. Several studies demonstrated there are genetic, genomic and proteomic differences between lethal and mild pathotypes. With the available Vna genomic resources including genome sequence and detailed transcriptome of the two strains, we aim to identify milRNAs and to elucidate their possible involvement in the pathogenesis process employing NGS sequencing and bioinformatics approaches. Small RNA samples of two Vna pathotypes were isolated from four different sources. Small RNA library was prepared and sequenced on the Ion Proton sequencing platform. Fungal milRNA precursors were predicted using several prediction tools showing MIReNA outperformed others. Predicted milRNAs were validated and candidates selected using criteria suggested for plant miRNAs. Several candidate milRNA precursors were selected for both pathotypes and are currently validated using stem-loop RT-PCR. Next step will involve determination of their endogenous targets and to investigate their role in infection process.

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1 – 16 Marc VALLS

Ralstonia solanacearum effectors expressed during potato infection

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Bacterial wilt of potatoes –also called brown rot- is a devastating disease caused by the vascular pathogen *Ralstonia solanacearum* that leads to significant yield loss. As in other plant-pathogen interactions, the first contacts established between the bacterium and the plant largely condition the disease outcome. In this work, we studied the transcriptome of the *R. solanacearum* strain UY031 early after infection in two accessions of the wild potato *Solanum commersonii* showing contrasting resistance to bacterial wilt. Total RNAs obtained from asymptomatic infected roots were rRNA depleted and deep sequenced and the bacterial reads were selected in silico and quantified. Although only 1-2% of the total RNAs corresponded to *R. solanacearum,* gene expression was detected for 4637 out of the 4778 annotated genes in strain UY031. Gene expression in planta was compared to the transcriptome obtained from bacteria grown in culture. We will present data on the Type Three Secretion System (T3SS) effectors and the metabolic pathways differentially expressed in potato and compare these results with previous studies carried out in tomato. This is the first report describing the *R. solanacearum* transcriptome directly obtained from infected tissue.

1 – 17 PIETER. J WOLTERS

Studying effectors from *A. Solani* and early blight resistance in potato

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The fungal pathogen *Alternaria solani* causes early blight in potato. Early blight is most severe on plants that are stressed by poor nutrition or drought, senescing plants and early maturing cultivars. The disease is an increasing problem and identification of natural resistance is of interest for the industry. So far, no potato cultivars have been identified that are fully resistant to early blight. To get insight into early blight resistance, we are studying effectors from *A. solani*. We sequenced the genome of *A. solani* using PacBio technology, resulting in a complete, gapless assembly of all 10 chromosomes. In addition, we performed RNAseq on RNA isolated from *in vitro* grown *A. solani* and infected plant tissue. Putative effector proteins from *A. solani* were predicted through comparisons with other *Alternaria* genomes, expression analysis and other selection criteria. These effectors will be subjected to functional tests in potato plants and their role in susceptibility or resistance to early blight will be studied. In order to identify sources of resistance against early blight, we screened a large collection of wild potato species (*Solanum* section *Petota*) and identified genotypes with high levels of resistance to *A. solani*. We are currently crossing susceptible and resistant genotypes for subsequent genetic mapping studies and to identify plant genes that can confer resistance against early blight.

1 – 18 **Jinbin WU**

A *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 type three secretion system (TTSS) effector targets and interrupts the SOBIR1-mediated signalling pathway

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Leucine-rich repeat receptor-like proteins (LRR-RLPs) and LRR-receptor-like kinases (LRR-RLKs) are cell surface receptors that are essential for detecting invading pathogens and subsequent activation of plant defence responses. In contrast to RLKs, RLPs lack a cytoplasmic kinase domain to trigger downstream signalling leading to host resistance. The LRR-RLK SOBIR1 constitutively interacts with Cf-4 and is required for Cf-4-mediated resistance to *Cladosporium fulvum*. Accumulating evidence shows that SOBIR1 is broadly required for RLP-involved resistance to fungal, oomycete and bacterial pathogens. *Pseudomonas syringae pv. tomato (Pst)* DC3000 injects approximate 30 effectors into host cells via the type three secretion system (TTSS) and causes bacterial speck disease on host plants. By using co-immunoprecipitation (co-IP) assays, we show that SOBIR1 is targeted by one of these effectors, independent of its kinase activity. Over-expression of *At*SOBIR1 causes a BAK1- and *At*SOBIR1 kinase activity-dependent auto-hypersensitive response (HR) in *Nicotiana benthamiana*. Interestingly, this auto-HR is specifically suppressed by the TTSS effector. Moreover, a mutant of the effector that loses its interaction with BAK1 and fails to suppress BAK1-mediated immunity, still interacts with SOBIR1. Further studies will be focused on the following questions; 1. Is the mutant effector still able to suppress *At*SOBIR1-induced HR? 2. As SOBIR1 is involved in Cf-4-mediated resistance to *C. fulvum*, are the effector and its variants able to suppress the Avr4-induced HR in *Cf-4* transgenic *N. benthamiana* plants. 3. Because SOBIR1 is targeted by bacterial effectors, is SOBIR1 required for resistance to *Pst* DC3000 in *N. benthamiana*?

Working Group 2

Plant proteins and processes targeted by effectors

2 – 1 Ahmed ABD-EL-HALIEM

Plant-Thrips arms race: Identification of Thrips Effector Proteins and their *in planta* Targets

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Thrips (*Frankliniella occidentalis*) is a major plant pest with a wide host range that imposes a substantial pressure on crop cultivations. Infestation of plants by thrips causes visible injuries leading to a decline in yield quality and quantity. These injuries are caused by thrips feeding, egg-laying and the reaction of plant tissue to injected thrips saliva. Thrips also serves as a viral vector in transmitting the tomato spotted wilt virus (TSWV) and the maize chlorotic mottle virus (MCMV). As a result of legislation demanding reduced pesticide usage, thrips resistance has become one of the most important breeding targets in vegetable crops. As a starting point towards the development of plants that are resistant to thrips we set out to identify thrips effector proteins released in the saliva during infestation. For this we utilized an integrated approach in which data was generated from whole-body and salivary gland transcriptomes and proteomics of thrips saliva. The generated data facilitated the prediction of thrips effectors using a custom-built bioinformatics pipeline. Thrips candidate-effectors are subsequently evaluated in tomato and pepper in a medium-throughput bioassay using a setup designed to reduce biological variation and increase data reproducibility. Identified effectors will be used to detect their *in planta* targets which will reveal information on the mechanisms of plant-thrips interaction and allow the development of thrips-resistant plants.

2 – 2 Nisha AGRAWAL

The *Sporisorium reilianum* effector Sad1 targets the maize RGLG2-like protein to suppress apical dominance in maize ears

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When the biotrophic smut fungus *Sporisorium reilianum* infects maize, it suppresses apical dominance of maize ears. The secreted fungal effector protein Sad1 is responsible for this phenotype but its mechanism of action is unknown. When expressed in *Arabidopsis thaliana*, GFP-Sad1 localizes to cytoplasm and nucleus and leads to multiple inflorescence branching. From a yeast two-hybrid screen, the maize E3 ubiquitin ligase RGLG2 was identified as one of the strongest interaction partners of Sad1. We show by BiFC that Sad1 interacts with RGLG2 at the plasma membrane in *Nicotiana benthamiana*. RGLG2 has a proposed myristoylation site at its N-terminus. Accordingly, interaction of RGLG2 with Sad1 in yeast two-hybrid experiments was only seen, when the C-terminal end of RGLG2 was used. RGLG2-GFP localized to the plasma membrane when expressed in *Nicotiana benthamiana*, but not when the N-terminal Glycine was mutated to Alanine. In *A. thaliana*, RGLG2 was shown to move from the plasma membrane to the nucleus upon stress, and there to function in dampening the stress response. We hypothesized that interaction of Sad1 with RGLG2 would interfere with RGLG2 function and lead to a prolonged stress response. Accordingly, we found that more H2O2 was present in floral tissue of plants infected with wildtype *S. relianum* than with *S. reilianum* Sad1 deletion strains. This suggests that Sad1 interacts with RGLG2 and that this interaction interferes with the activity of RGLG2 in dampening the stress response in maize ears. This in turn might lead to the observed phenotype of suppression of apical dominance.

2 – 3 Philip ALBERS

The immune kinase PBS1 phosphorylates a remorin protein which is also targeted by the bacterial effector HopZ1a

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The bacterial type-III-secreted effector HopZ1a from *Pseudomonas syringae* was shown to possess acetyltransferase activity against tubulin and JAZ transcription factors leading to a suppression of plant immunity. Using a yeast-two-hybrid screening, we identified a novel target protein of HopZ1a in the model organism *Nicotiana tabaccum.* The protein we identified belongs to the remorin family of plasma membrane proteins, and we named it HopZ1a interacting remorin 1 (HIR1). To characterize the role of HIR1 in plant defense, we performed additional Y2H screens and identified the immune kinase PBS1 as putative HIR1-interacting protein. Using co-IP assays we proved the interaction of HopZ1a and HIR1, as well as PBS1 and HIR1 *in vitro*. Split-YFP experiments confirmed the interaction in planta with both complexes associating at the plasma membrane. Preliminary results indicate that overexpression of HIR1 leads to increased basal PTI marker gene expression. Using *in vitro* kinase assays we confirmed that HIR1 is a direct phosphorylation target of PBS1, and MS-MS data show that HIR1 is phosphorylated by PBS1 at S64. Mutating S64 and S65 to alanine reduce the phosphorylation signal in radioactive kinase assays confirming that PBS1 phosphorylates HIR1 at the regulatory N-terminus, but also suggesting the existence of additional phosphorylation sites. In summary, our findings support the hypothesis that HIR1 might act in a complex together with PBS1 during PTI and hence is targeted by HopZ1a to manipulate immune signaling.

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2 – 4 Maria ERCOLANO

Integrate –omics approaches for undercovering tomato tuta absoluta interaction

Manzo D., Garonna A, D'Esposito D., Pennacchio F., Lanzooti V.,Frusciante L. Ercolano MR

Tuta absoluta is one of the most destructive chewing insects in tomato crops causing near to 70% yield losses. Despite its economic importance, little is known about the molecular basis of the interaction between this leafminer and tomato plants. To investigate the tomato response to T.absoluta challenge , a multi-omic approach was carried out. Tolerant and Susceptible cultivated tomato genotypes as well as the derived F1 hybrid were employed to shed light on the plant response to the herbivore feeding. RNA sequencing of infested and not infested genotypes revealed thousands of differential expressed genes (DEGs) between the two conditions in the three genotypes. The Gene Ontology (GO) enrichment analysis showed several over-represented categories, including responses to biotic stress and external stimulus, response to wounding and signal transduction and transmission in tolerant genotypes. The NMR-approach metabolome analysis of the three infested lines showed consistent differences in the metabolic profiles between exposed lines and their respective controls. Compressively our results suggest a chemical shift in the no-infested lines, with the involvement of secondary metabolites and terpene production. Moreover, the integration between –omics approaches showed to be useful for managing gene target selection during tomato crop improvement.

2 – 5 Johannes FAHRENTRAPP

Gene expression in tomato leaves during pathogen infection

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Plants are mostly non-hosts, and thus resistant, to most potentially pathogenic organisms. Plant reaction to pathogen attack is subdivided into the general response called pathogen triggered immunity (PTI) and the disease specific effector triggered immunity (ETI). If the invaded plant tissue can identify its pathogen the reaction to and the interaction with pathogenic organisms of susceptible tomato leaves (*Solanum lycopersicum* Heinz1706 and Moneymaker) may be distinct dependent on the infecting pathogen, the disease incubation time and the location of infection within the leaf. We investigated the interaction of late blight, *Phytophthora infestans*, powdery mildew, *Oidium neolycopersici*, and gray mold, *Botrytis cinerea*, by means of RNA-seq aiming to identify genes being specifically expressed for each disease in our experimental setup. A selection of gray mold- and late blight-specific differentially expressed genes was tested for their temporal and spatial expression and its potential use in disease detection. When infecting only the terminal leaflet of the tomato compound leaf, some of these genes were stronger upregulated at infection site and the rachis of the compound leaf (e.g. *DA1-related 1-like*) compare to other genes which were stronger upregulated in the adjacent leaflets (e.g. a Beta-1,3-glucanase). During the first 48 hours the upregulation of disease specific genes was approximately four times stronger during *B. cinerea* with respect to *P. infestans*.

2 – 6 Jernej JAKSE

In-silico prediction and validation of potential gene targets for viroid derived small RNAs in hop plants

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Viroids are the smallest known plant pathogens of economic importance, characterized by extremely small covalently closed circular genomes with strong rod-like secondary structures. Four different viroid species have been confirmed as being able to infect hops, which makes hop an attractive model plant for studying viroid-plant interactions. How these molecules without coding for any proteins are able to induce severe disease symptoms in plants is still under investigation but current evidence suggests that the host defense response produces enormous amounts of viroid-derived small RNAs (vdsRNAs). In the proposed model, these vdsRNAs use host RNA silencing machinery to target host mRNAs, which eventually trigger the symptoms. A computer based approach was undertaken to search for possible targets for HLVd and CBCVd vdsRNAs in hop's Illumina transcriptome. All possible viroid's 21, 22 and 24 bp mers were aligned against the transcriptome using RNA-target identification tools. In total, 2,041 possible targets for the action of vdsRNA were revealed and annotated. Further selection of targets for the RT-qPCR experiment was performed including transcripts involved in hormonal pathways of plant response, transcripts targeted by vdsRNAs that are present in plants in extremely high concentrations and transcripts showing highest possible match between the vdsRNA. Selected pathogenesis related (PR) genes expression was monitored as well. Both groups of viroid infected plants (HLVd, CBCVd) showed strong up-regulation of PR genes compared to viroid free plants, while other studied genes showed either up- or down-regulation. The work is the first attempt to elucidate interactions between vdsRNAs and hop genes.

2 – 7 Marek KOTER

The cornucopia of isomiRs tunes host response to *Globodera rostochiensis* parasitism

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Plant Parasitic Nematodes induce deep changes in genes expression of their hosts. Increasing number of reports suggest the active role of different classes of miRNA in this process. We studied tomato root miRNome dynamics in response to *G. rostochiensis* using miRNA-Seq on Illumina platform. We detected 4270 novel miRNAs sequences using miRCat software and their expression in three time points was analyzed using edgeR package. The highest miRNA complexity was detected in early stages of syncytium development. A huge number of isomiRs were detected by IsomiRage software showing predominant variation on 3' end. Putative target mRNA sequences for isomiRs were detected by psRNATarget software showing incidentally different regulatory potential of miRNA variants. Discovering the potential role of isomiRs will contribute to better understanding of complicated post transcriptional control of gene expression.

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2 – 8 Tjasa LUKAN

Promoter analysis of several genes involved in plant immune response

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Salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) signaling pathways represent the backbone of the defense signaling network, with other hormonal signaling pathways feeding into it. The importance of SA, JA and ET as dominant primary signals in local and systemic induced defense signaling has been well documented. However, the way these signal molecules function in a complex network of interacting pathways is less well understood and the majority of research has been done on model plant species and very little on potato. Our aim is to upgrade our current understanding of the roles of these three hormones in the plant's immune system, focusing on promoter analysis of several genes involved in plant immune response. Promoters of several genes from signaling pathways have been amplified from different potato cultivars, sequenced and compared to the available model genome sequences. Obtained sequences were analysed with three different databases for detection of plant cis-acting regulatory DNA elements and transcription factors: TRANSFAC, PlantCARE and PLACE. The results showed that promoter sequences of the same gene differ between cultivars. Furthermore, each gene can have different promoter sequences within the same cultivar. Using different databases we managed to characterized transcription factor binding sites and established how transcription factor binding sites vary between promoters of the same gene within each cultivar.

2 – 9 Mateusz MATUSZKIEWICZ

Complex PCD regulation during compatible plant-nematode interaction

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Plant parasitic nematodes are common pests of many crops causing substantial losses in agriculture. Infective larva invade roots and secrete protein cocktail containing many effectors. These secretions suppress the defense response and trigger developmental and metabolic reprogramming of cells leading to syncytium formation. Depending on the R-gene composition nematode secretions may evoke the PCD at different intensities including HR in the case of avirulence. During compatible interaction however pro-PCD signals still seem to be important. Changes in the distribution of the salicylic acid, excessive accumulation of reactive oxygen species during infection lead to the death of plant cells. In order to shed more light on the role of PCD in plant nematode interaction several regulators of PCD were analyzed. Mutants of genes belonging to LSD1 regulon were checked during infection tests. Usually changes in feeding structure size were observed, whereas the susceptibility was much less variable. The negative regulator of PCD - LSD1 and positive - LOL1 and LOL2 showed slight but antagonistic patterns of susceptibility to *Heterodera schachtii*. The observations of the nematode growth development and ultrastructure of syncytia induced in *lsd1* lines revealed retarded growth, smaller vacuoles and electron translucent cytoplasm. To combine morphological analysis of *lsd1* mutant with molecular regulation of PCD RNA-seq analysis on *H. schachtii* infected and uninfected roots was done. Inactivation of LSD1 dramatically decreased complexity of reaction to nematodes (from 3007 to 1199 differentially transcribed genes). These results demonstrate that PCD is highly regulated process in compatible plant-nematode interaction. This work was supported by National Science Centre (grants no. 50130041000M0007799).

2 – 10 Sergio MAURO

Proteomic study of SUMOylation during *Solanum tuberosum / Phytophthora infestans* in (in-) compatible interactions

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Invasive plant pathogens have developed the ability to modify metabolic processes of their host and to promote those which facilitate their growth at the expense of the host. SUMOylation is widely conserved posttranslational modification in *eukaryotic* organisms, and this process exerts pleiotropic functional consequences that include changes in many aspects of protein activities, functions, stability, localization and interactions with other partners. Here we provide experimental evidence indicating that the SUMO pathway is partially under transcriptional control in *Phythopthora infestans* infected *Solanum tuberosum* leaves. We also report the results of using a recently developed proteomic approach based on the use of 3D gels to identify the endogenous SUMO targets and we show that during the infection process the abundance of most of these conjugates either increases, or decreases, significantly. This study primes further investigations into the molecular connections between host SUMOylation and Phythopthora infestans infection.

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2 – 11 Oliver NAGEL

Impact of *Xanthomonas* type III-effector proteins on the transcriptome of tomato

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Type III-effector proteins (T3Es) are essential virulence factors of *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*), which are translocated directly into the cytoplasm of plant cells. Once inside the plant cell, T3Es interfere with plant proteins or DNA, to alter cellular processes to the advantage of *Xcv*. Using microarray-analysis, we studied transcriptome changes of *S. lycopersicum* leaves after infection with the wild type strain and T3SS-deficient strain *Xcv*85-10Δ*hrcN*, respectively. 2,856 of 34,383 annotated *S. lycopersicum* genes were altered in their transcript abundance and are presumably involved in plant defense responses. In case of the infection with the wild type strain *Xcv* 85-10, the transcript abundance of 2,035 *S. lycopersicum* genes was altered. The transcript abundance of an intersection of 968 genes was both, altered during plant defense responses during infection with *Xcv* 85-10Δ*hrcN* and T3E-dependently altered in the opposite direction. The majority of these genes are involved in plant defense responses. In addition, many genes play a role in hormone perception and -biosynthesis, signal transduction and photosynthesis. The up- or downregulated tomato genes identified in our study provide a foundation for further studies to elucidate the contribution of selected T3Es to transcriptome changes.

2 – 12 Cyrus Raja Rubenstein SABBAGH

Towards a global interactomic map between the *Ralstonia solanacearum* species complex core type 3 effectors and the tomato proteome

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

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The *Ralstonia solanacearum* species complex (RSSC) virulence strategy relies majorly on a spectrum of type 3 effectors (T3Es) injected inside the plant cells *via* a Type 3 Secretion System (T3SS). Thanks to the currently known genomic sequences of the different plant pathogenic strains of the RSSC, our group was able to identify a list of 30 conserved or "core" T3Es. As these core-T3Es have been conserved through the evolution of the highly diverse RSSC, with strains affecting different host plants, they must be important for virulence. Each core T3E representative was cloned from the GMI1000 strain (*R. pseudosolanacearum*) and further sub-cloned in the two yeast-2-hybrid plasmids: Prey and Bait constructs. I will be presenting our efforts towards the identification of putative tomato targets for all of these 30 core-T3Es. This will be carried out by systematic yeast-2-hybrid screening against a tomato root cDNA library. As our goal is to provide a map of all the detectable core T3E-tomato targets, we will also test by matrix pairwise yeast-2-hybrid all identified tomato targets against all available T3Es (core and non-core T3Es). The ultimate goal of this resource is to identify plant proteins and signaling pathways targeted by these effectors to guide our future efforts towards control strategies.

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2 – 13 Heike SEYBOLD

Actio *est* Reactio? Successful bypassing of wheat immune responses by a fungal pathogen

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The plant immune system is a successful cooperation of general boundaries and specific responses on molecular and transcriptional levels preventing the plant from invasion of harmful pathogens. Still, specialized pathogens have found ways to overcome the complex immune machinery of their hosts. The ascomycete pathogen *Zymoseptoria tritici* is able to infect susceptible varieties of wheat (*Triticum aestivum*). So far little is known about the physiological responses of wheat to infection by *Z. tritici*. Yet understanding how this pathogen compromises host immune responses is essential to improve resistance breeding. In this context, the onset as well as the regulation of lifestyle switches is of special interest as *Z. tritici* is described as a hemibiotrophic pathogen for which the timing of lifestyle changes determines the successful host invasion. Detection of reactive oxygen species (ROS), measurement of cell wall components and monitoring of chlorophyll content are examples of the methods used to study the progress of the infection. Comparisons of susceptible and resistant wheat cultivars will further increase our knowledge about the evolution of potentially essential and unique virulence factors between different *Z. tritici* isolates and wild relatives of the wheat pathogen.

2 – 14 Suayib USTUN

How plant pathogenic bacteria co-opt the proteolytic degradation pathways

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Autophagy and the ubiquitin- proteasome system (UPS) are the major pathways for protein degradation in eukaryotes. They orchestrate many cellular processes during development and in response to environmental stimuli such as microbial infections. In plants, the contribution of the UPS to immune responses and its targeting and exploitation by pathogens are well documented, but the role of autophagy remains elusive. Regarding the UPS we could show that the proteasome acts as a hub during local and systemic defense responses upon *Pseudomonas* infection in *Arabidopsis thaliana*. In addition, we provide evidence that *Pseudomonas* inhibits proteasome activity in a type-III secretion dependent manner, mainly mediated by type-III effector protein (T3E) HopM1. Biochemical characterization of HopM1 by mass-spectrometry indicates that HopM1 interacts with several UPS components. As recent evidence suggests that the proteasome is degraded by autophagy upon pharmacological inhibition of proteasome activity, we will elucidate whether *Pseudomonas* exploits autophagy to modulate proteasome function. Thus, a systematic screening method has been established to screen for T3Es potentially affecting autophagy. Recent advances how *Pseudomonas* probably alters autophagy responses to inhibit proteasome activity will be presented and discussed.

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2 – 15 Patrycja ZEMBEK

The function of the N-terminus of HopQ1 effector from *Pseudomonas syringae*

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HopQ1 (for Hrp outer protein Q) is a type III effector that has been recently acquired by *Pseudomonas syringae* strains. Sequence analysis of the HopQ1 effector family revealed low amino acid diversity among bacterial strains. The amino acid substitutions were confined mostly to a disordered region in the N-terminus of HopQ1 that precedes the predicted nucleoside hydrolase domain. However, two strain-specific variants of HopQ1 from *phaseolicola* 1448A (Psph) and *tomato* DC3000 (Pst) pathovars, that differed only at six amino acid positions, showed considerable differences in their ability to suppress plant defenses. To pinpoint the residues that determine this process we generated a set of HopQ1 variants with substitutions at the respective amino acid positions. Preliminary results showed distinct patterns of nucleocytoplasmic partitioning of those variants within plant cells. We noticed also that the variants were diversely susceptible to proteolytic cleavage by an unknown plant protease. The PstHopQ1 variant with the substitutions S87L_L921R recovered the ability to inhibit MAPKs' activities in Arabidopsis protoplasts. Interestingly, these mutations abolished also the susceptibility to the proteases suggesting that the cleavage might regulate the function of the effector in plant cells. We have previously shown, that the N-terminus of HopQ1 contains the 14-3-3 binding motif. Collectively, our previous and current studies suggest that the unstructured N-terminal tails of the type three effectors besides their involvement in the secretion process may play an important regulatory role.

Working Group 3

Effector evolution and diversification

3 – 1 Norfarhan BINTI MOHD ASSAAD

Genome-wide association mapping identifies multi-locus resistance evolution to fungicides in the barley pathogen *Rhynchosporium commune*

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Rapid evolution of plant pathogens is a major concern for sustainable food security in agro-ecosystems. Fungicide application is an integral component to protect yields, however pathogen populations readily evolved resistance to most commonly applied fungicides. Resistance towards azole fungicides is frequently caused by mutations in the gene encoding a protein of the ergosterol biosynthesis pathway (*CYP51*). However, some fungi gained azole resistance independently of *CYP51* mutations and the mechanisms leading to *CYP51*-independent resistance are poorly understood. We re-sequenced 120 isolates of *Rhynchosporium commune*, the causal agent of the barley scald disease collected from 9 worldwide populations and performed an unbiased screen of azole resistance loci using genome-wide association studies (GWAS). We found that mutations in highly conserved genes encoding the vacuolar cation channel YVC1, a transcription activator, and a saccharopine dehydrogenase made significant contributions to fungicide resistance. These three genes were not previously known to confer resistance in plant pathogens. However, YVC1 is involved in a conserved stress response pathway known to respond to azoles in human pathogenic fungi. We also performed GWAS to identify genetic polymorphism linked to fungal growth rates. We found that loci conferring increased fungicide resistance were negatively impacting growth rates, suggesting that fungicide resistance evolution imposed costs. Analyses of population structure showed that resistance mutations were likely introduced into local populations through gene flow. This study shows that fungal pathogen populations can rapidly evolve a complex genetic architecture for fungicide resistance within a short time span.

3 – 2 Beatrice CORSI

Using Multiparent Advanced Generation Inter-Cross (MAGIC) populations to investigate wheat leaf spot group (LSG) host–pathogen interactions in the UK

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The wheat leaf spot group (LSG) of necrotrophic pathogens (*Parastagonospora nodorum* - *Pn*, cause of Septoria nodorum blotch, *Zymoseptoria tritici* - *Zt*, Septoria tritici blotch and *Pyrenophora tritici-repentis* - *Ptr*, tan spot) induce major wheat yield losses in Europe. The implementation of effective genetic disease resistance represents an important route towards increasing sustainable wheat production. As part of the ERA-CAPs funded project 'EfectaWheat' we are using an 8-parent Multi-parent Advanced Generation Inter-Cross (MAGIC) genetic mapping population, genotyped with an Illumina 90k single nucleotide polymorphism (SNP) array, to investigate the genetics of LSG resistance in wheat. Representative UK *Pn* and *Ptr* isolates were used to inoculate MAGIC field trials undertaken at NIAB (Cambridge, UK) in 2016. Disease severity was scored, as well as additional phenotypic data, including plant height and heading date. The population has previously been screened at the seedling stage for sensitivity to known *P. nodorum* and *P. tritici-repentis* effectors. The combined datasets will be used in genetic analyses to identify QTLs and possible relationships between wheat genetic loci controlling effectors- and pathogen-mediated host resistance/susceptibility. Ultimately, this information will provide useful tools for the development of improved wheat varieties with increased genetic resistance to LSG pathogens.

3 – 3 Marta GRECH-BARAN

Natural variation of HopQ1 TTSS effector from *Pseudomonas syringae*

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HopQ1 is a type three effector secreted by many strains of *Pseudomonas syringae*, a gram-negative bacterium that infects a wide range of plants. We analyzed bacterial strains pathogenic to various stone fruit trees, to find amino acids involved in strain-specific functions of this effector. HopQ1 homologs were found in 67 strains of *P. syringae* pv. *morsprunorum* while we were not able to detect their presence in any of *P. syringae* pv. *syringae* strains. Sequence analyses revealed two basic HopQ1 classes. The pattern of their distribution fully correlated with the two known races of *P. syringae* pv. *morsprunorum*, that is race 1 that infects sweet cherry and plum and race 2 that infects sour cherry trees. This implicates that the differences between HopQ1 homologs may be a consequence of their adaptation to various hosts. Furthermore, the co-occurrence of HopQ1 and HopR1 suggested that these two effectors could co-operate during the infection process. To test this hypothesis we constructed plasmids with operons encoding combinations of HopQ1 and HopR1 variants derived from two strains. We next introduced the plasmids to a strain deficient in 28 effectors and test the ability of the transformants to induce hypersensitive response. Our preliminary experiments show that the co-expression of HopR1 from the same strain masks the recognition of HopQ1 by plants. In contrast, when two effectors from different strains were co-expressed, HopQ1 triggered plant defense response. This suggests that variation of HopQ1 may also reflect the fact that the effectors are tailored to cooperate with each other.

3 – 4 Yogesh GUPTA

Field dynamics and patho-genomics of Asian soybean rust pathogen *Phakopsora pachyrhizi*

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Soybean (*Glycine max*) is an important legume crop and a rich source for plant proteins and vegetable oil. A major constraint for soybean production is Asian soybean rust (ASR) which causes yield losses of up to 80%. ASR is caused by the obligate biotrophic fungus *Phakopsora pachyrhizi* and can only be controlled by fungicide application. Extensive efforts have been made to find genetic resistance against *P. pachyrhizi* however, the pathogen has rapidly overcome major resistance genes *Rpp1-5.* Remarkably, *P. pachyrhizi* infects over 153 different legume species under suitable conditions, which suggests that *P. pachyrhizi* maintains diverse pathogenicity factors. The degree and distribution of genetic variation in the pathogen population is key for informed deployment of novel resistance genes but is largely unknown for *P. pachyrhizi.* To understand the field variation and population structure of *P. pachyrhizi*, infected field samples of soybean are collected from different locations across Brazil and east Africa. We have developed a next-generation sequencing approach to explore the population structure and genetic variation of *P. pachyrhizi* in these very different geographic locations. Sequencing of field samples will allow us to study sequence polymorphisms in *P. pachyrhizi*. We will aim to obtain a detailed understanding of the effector diversity in the field population of *P. pachyrhizi*. We will use this information for the sustainable deployment of genes that confer resistance to Asian Soybean Rust in transgenic soybean.

3 – 5 Tina JORDAN

The TAL effectors *avrBs3* and *avrBs4* are widespread in *Xanthomonas euvesicatoria* field strains, but show limited diversity

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TAL (transcription activator-like) effectors are known to contribute to virulence in numerous *Xanthomonas* species. This group of proteins interacts with host DNA and induces the transcription of so-called susceptibility genes. *Xanthomonas euvesicatoria* (*Xe*) is the causal agent of bacterial spot in pepper. On EU territory yield losses up to 30% due to bacterial spot were reported and in Spain bacterial spot is considered a major pathogen on pepper. Unlike other Xanthomonads, *Xe* strains only carry two TAL effectors: *avrBs3* and *avrBs4*. To determine sequence diversity and geographical distribution of these two effectors we analyzed 73 strains from various regions and time periods. Our analysis revealed that *avrBs3* and *avrBs4* are widespread in field populations of *Xe*. Only seven strains did not carry any TALE, and half of the strains carried both. Despite the repetitive and modular structure of the DNA binding domain, that could favor recombination events we found very little sequence diversification. So far, we found only 15 variants of AvrBs3 and AvrBs4. Most common variations are RVD changes. In conclusion these results indicate an important fitness contribution of these two proteins in *Xe* under field conditions.

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3 – 6 Pezhman SAFDARI

A genomic approach to unravel plant-pathogen coevolution in the wild

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Due to relatively well studied role of effector proteins and established bioinformatics methods to identify them in fungal genomes, effector proteins are a suitable means to investigate the genetic basis of infectivity traits in pathogens. The interaction between *Plantago lanceolata* and its fungal pathogen *Podosphaera plantaginis* offers unique opportunities for linking effector variation to infectivity traits and epidemiological dynamics. The key aim of this study is to identify candidate genomic loci in P.plantaginis associated with infectivity traits and analyze these for the presence of candidate effectors and to quantify variation at these loci from an existing extensive sample database.

3 – 7 Jadwiga SLIWKA

Sequence diversity and expression of *AvrSmira1* and *Avr-vnt1* effectors of *Phytophthora infestans* in virulent and avirulent isolates

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Potato resistance genes *Rpi-Smira1* and *Rpi-phu1/Rpi-vnt1* provide efficient defence against the most important potato disease, i.e. late blight caused by *Phytophthora infestans*. Both genes have not been, so far, widespread in potato fields, except the experimental ones, and they are currently being introduced into many breeding programs. In an attempt to predict the durability of resistance of the future cultivars with the *Rpi-Smira1* and *Rpi-phu1* genes we studied the diversity of corresponding effectors. Virulence of over 300 recent Polish *P. infestans* isolates was tested in detached leaflet assays using cultivar Sárpo Mira and plants with the *Rpi-phu1* gene. Frequency of virulent isolates was 30% in case of cultivar Sárpo Mira and 3% on plants with the *Rpi-phu1* gene. DNA sequences of *AvrSmira1* and *Avr-vnt1* effectors were obtained for 96 *P. infestans* isolates of various origins and indicated bigger diversity for *AvrSmira1* as well as no clear correlation of any effector sequence to the virulence data. Expression of *AvrSmira1* and *Avr-vnt1* effectors was measured by qPCR in relation to *elongation factor 2-α* expression level in detached leaflet assays and in the samples from a field trial. The *Avr-vnt1* effector expression was not switched off permanently in virulent isolates to avoid recognition by an R protein, but reappeared in a post-biotrophic phase and was present constantly when infecting plants without the corresponding *R* gene.

3 – 8 Cécile THOMAS

Life-history traits of *Phytophthora infestans* condition the effectiveness of resistance induced by PAMPs in potato.

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The recognition of pathogens *via* pathogen-associated molecular patterns (PAMPs) induces general defense responses (PAMP-triggered immunity) in plants. Subsequently, pathogens can acquire effectors to decrease or suppress defense activity, allowing host colonization (Effector-triggered susceptibility). We previously demonstrated that a Concentrated Culture Filtrate of *Phytophthora infestans* (CCF) primes defense reactions in potato (Saubeau *et al*, 2016), but the metabolites produced decrease pathogen growth *in vitro* but not *in vivo* (Kröner *et al.*, 2012). We postulated that *P.infestans* could be able to escape or modulate the activity of defense metabolites. We tested this hypothesis in two independent experiments on a susceptible potato cultivar (BF15). Plants were sprayed with CCF and inoculated 48h later with four strains of *P.infestans* differing for lesion growth rate. The induction of genes involved in different metabolic pathways was analyzed with qRT-PCR in leaf samples sprayed or not with CCF. Pathogen development was characterised by measuring necrosis area, quantifying mycelium DNA *in planta* and counting sporangia on infected leaflets. Our results showed that after induction, the necrosis area decreased only for the two fastest growing strains until four days post-inoculation (dpi), while sporangia production decreased only for one slow strain. DNA quantity increased for three strains until 5dpi, although it significantly decreased at 5dpi for a slow strain. Our results therefore suggest that efficiency of induced resistance depends on life-history traits of each strain, notably *in vivo* growth. It also probably depends on the effectors involved in the interaction. We now investigate whether effector induction is also strain dependant.

3 – 9 Maria Carlota VAZ PATTO

Portuguese common bean landraces vs. *Fusarium oxysporum*: a diverse array of resistances (first steps into an unexploited source for resistance breeding)

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Common bean (*Phaseolus vulgaris* L.) is the most important food legume worldwide. Portugal holds a very diverse common bean germplasm consisting of landraces that resulted from more than five centuries of natural adaptation and farmer's mass selection, not yet fully explored in breeding. Fusarium wilt is among the diseases that cause major yield losses in this crop. The agronomical, morphological and molecular diversity observed in this Portuguese common bean germplasm anticipates the presence of diverse sources of resistance not yet explored in breeding and foresees the identification of novel resistance / effector target genes. In order to identify alternative sources of resistance for future allelic diversity breeding exploitation of potential resistance genes, we characterized the resistance to fusarium wilt of 150 Portuguese common bean landraces. Evaluation took place under controlled conditions. Seven-day-old seedlings were inoculated with *Fusarium oxysporum* f. sp. *phaseoli*, and symptoms assessed every three days, from 7th to 30th dai, using a visual disease index scale from 1 (healthy leaf) to 5 (dead leaf). These data were used to calculate the AUDPC (area under the disease progress curve) values. Infection responses revealed great variability among landraces, with the identification of an array of diverse sources of resistance. Representatives of the different levels of resistance were selected for a future more detailed molecular characterization of the resistance mechanisms. Once these mechanisms are characterized, this germplasm can be better exploited on common bean resistance breeding.

3 – 10 Ronja WONNEBERGER

Identification of resistance to *Drechslera teres* in Norwegian barley and population structure of a Norwegian *D. teres* population

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The fungal pathogen *Drechslera teres* is the causal agent of net blotch disease in barley which can cause grain shriveling and yield loss. Resistance of most current commercial Norwegian cultivars is insufficient. This study aims at detecting QTL associated with resistance and susceptibility in the germplasm most relevant to Norwegian barley breeding. Together with knowledge of the genetic profile of the Norwegian net blotch population, we will be able to optimize resistance breeding in barley. Resistance to three Norwegian *D. teres* isolates was assessed in a segregating biparental cross of the Norwegian cultivars Arve and Lavrans and an association mapping panel in seedlings in the greenhouse and in adult plants in the field. In the biparental population, QTL mapping revealed a major QTL on chromosome 5H stable in all environments, which explained up to 55% of the genetic variation. Eight additional QTL explained up to 17% each, and one of them was isolate-specific. Association mapping in 209 Nordic barley lines revealed 15 QTL, each explaining less than 15%. QTL on 3H and 6H were found both in seedlings and adult plants. KASP (Kompetitive Allele Specific PCR) markers for these major QTL will be developed and implemented in Norwegian barley breeding programs. Additionally, 365 Norwegian *D. teres* isolates and a selection of globally collected isolates were ddRAD genotyped to obtain SNP markers to study the genetic diversity and population structure of the current Norwegian fungal population. This data will also allow us to perform Genome Wide Association Studies (GWAS) to identify potential novel virulence genes.

Working Group 4

Immune receptors and allelic variants of host targets for resistance breeding and engineering

4 – 1 Carolina AGUILERA-GALVEZ

Recognition specificity of AVR2 effectors from *P. infestans* in *Solanum* native of Mexico and Peru

Carolina Aguilera-Galvez[1], Nicolas Champouret[1], Hendrik Rietman[1], Doret Wouters[1], Jonathan D.G. Jones[2], Richard G.F. Visser[1], Vivianne G.A.A. Vleeshouwers[1]

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Late blight, caused by the oomycete *Phytophthora infestans* is the most devastating disease for potato. A common strategy to control the disease is the introgression of resistance (*R)* genes in potato cultivars. However, single-dominant *R* genes have rapidly been defeated, due the genome plasticity of *P. infestans*. Nowadays, simultaneous deployment of multiple broad spectrum *R* genes is advised. In this study, we are exploiting effectoromics to identify new *R* genes. We focus on AVR2 effectors, a highly diverse family of 14 members with some level of similarity in the C-terminal effector domain. A wide collection of wild *Solanum* section *Petota* species were screened for responses to AVR2 effectors. Cell death responses were identified in *Solanum* native of Mexico and Peru. In Mexican *Solanum* species, AVR2 members are recognized by the R2 protein. However, the recognition specificity in South American *Solanum* species is mediated by different *R* genes. We isolated and functional characterized a new resistance gene from *Solanum huancabambense* (*Rpi-hcb1*). *Rpi-hcb1* shows a differential recognition of AVR2 effectors compared to *R2* and confers resistance to *P. infestans*. Ultimately, we aim to exploit and pyramid *R* genes with complementary recognition spectra to achieve a more broad-spectrum resistance to *P. infestans*.

4 – 2 Balazs BARNA

Some aspects of host and nonhost interactions of powdery mildews

Balazs Barna

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Powdery mildews are one of the most devastating pathogens of crops all over the world. Some of these typical biotrophs have very narrow host specificity, like the cereal powdery mildew *Blumeria graminis*. Others, as *Golovinomyces orontii* colonize many dicotyledonous plant species; like tobacco and *Arabidopsis thaliana* plants. There are major resistance genes against powdery mildew in cereals, while tobaccos are all susceptible to powdery mildew, except a Japanese domestic tobacco cultivar, in which resistance is due to aberrant splicing of MLO orthologues. It is noteworthy that until now no confirmed data can be found on powdery mildew infection on the monocot model plant *Brachypodium distachyon*. In our experiments near-isogenic lines of barley cultivar Pallas without or with Mla, Mlg and mlo resistance genes, wheat cultivar Winzi, *B. distachyon* diploid inbred lines *Bd21 and Bd 21-3,* as well as NahG, a salicylic acid deficient transgenic tobacco and its Xanthi control lines were used in addition to *B. graminis* f. sp. *hordei* (*Bgh*) and *tritici* (*Bgt*), as well as *G. orontii* tobacco and *Arabidopsis* isolates. The highest increase in ion leakage, antioxidant activity and ethylene production was shown in compatible interactions. Electrophoretic RNase enzyme patterns of barley, wheat and *B. distachyon* were very different from each others and activity was induced even in nonhost plants. Interestingly, PR-1b, PR-1c, WRKY1 and WRKY12 gene expressions were upregulated also in the compatible tobacco-powdery mildew interactions. Pre-treatment of leaves with protein kinase inhibitor staurosporine or protein phosphatase inhibitor okadaic acid inhibited all powdery mildews.

4 – 3 Aleksandra BIALAS

Evolution and specialization of an NLR-integrated domain

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In order to successfully colonize plants, pathogens secrete effectors that manipulate host physiological processes and promote infection. The rice blast fungus Magnaporthe oryzae targets heavy metal-associated (HMA) domain host proteins. However, these domains have also integrated into two rice NLR immune receptors, Pik-1 and RGA5, where they bait the M. oryzae effectors AVR-Pik, AVR-Pia and AVR1-CO39, respectively, to activate disease resistance. We hypothesize that during rice evolution, these NLR proteins acquired a domain from an effector target, i.e. an HMA domain, to gain a new pathogen sensor activity, and hence expand the plant's capacity to mount an immune response against pathogens. The objective of this study is to reconstruct the evolution of the integrated HMA domain, and test hypotheses about adaptive evolution of an NLR with an integrated domain. We discovered that Pik-1 and its partner NLR Pik-2 have orthologues in other Oryza species, indicating that HMA integration into Pik-1 occurred at least 20 million years ago. We performed genetic and biochemical analyses to determine the evolutionary dynamics of Pik-1 binding and response to the AVR-Pik effector. Moreover, we used phylogenetic analyses to reconstruct the ancestral sequence of the integrated HMA domain, and assayed the resurrected HMA in planta for the effector binding. Our preliminary results revealed that Pik-1 HMA domain evolved towards higher binding affinity to M. oryzae effector AVR-PikD. Next, we aim to further understand HMA domain evolution in the context of intramolecular coevolution with the NLR chassis.

4 – 4 James COCKRAM

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

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EfectaWheat is a recently funded project under the ERA-CAPS second 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 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, are being 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.

4 – 5 Gulay DAGDAS

Hotspots of NLR receptor diversification and evolution of NLR-kinases in wheat

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Previously, we mined genomes of over 40 plant species to characterize R-genes of NBS-LRR class (NLRs) as well as NLRs with additional integrated domains (NLR-IDs). With more detailed analyses of NLR-IDs in wheat, we uncovered a particular clade that is more prone to generating diverse NLR-IDs. The integrated domains include a well-characterized HMA domain, as well as protein kinases (PKs), phosphatase, DNA binding factor B3, Glutaredoxin and Exocyst subunit, Exo70. Most importantly, association of this NLR clade in wheat with diversity of integrated domains suggests that it can function as a plant immune receptor platform that is more amenable to gene fusions than other clades. Kinases are essential regulators of plant innate immunity. The phylogenetic analyses NLR-PKs, known PKs targeted by pathogen-derived effectors and kinases with a role immunity and their homolog in wheat indicate that (i) NLR-PK fusions cluster with reference kinases which allows to predict NLR-PK/effector pairs, (ii) NLR-PKs are expanded in *Triticeae* and (iii) some NLR fusion clades include genes from all wheat types (progenitor wheat AA genome and DD genome, pasta wheat, bread wheat) implicating that some fusions are ancient events.

4 – 6 Rowena DOWNIE

Mapping the wheat *Snn3-B1* locus conferring sensitivity to the *Parastagonospora nodorum* necrotrophic effector SnTox3 using an eight founder multi-parent advanced generation intercross (MAGIC) population and the Avalon x Cadenza doubled haploid population.

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Parastagonospora nodorum is a necrotrophic fungus and a pathogen of one of the world's most economically important cereal crops, wheat (*Triticum aestivum* L.). This important pathogen mediates host cell death using proteinaceous necrotrophic effectors, and this is believed to provide the nutrients that allow the infection process to continue. Pathogen effectors have allowed intricate host genetic resistance mechanisms to be separated into constituent elements. In *P. nodorum*, *SnTox3* encodes a cysteine-rich 17.5 kDa mature proteinaceous effector, SnTox3. Sensitivity to SnTox3 is conferred by *Snn3-B1* and *Snn3-D1* located on wheat chromosomes 5BS and 5DS, respectively. Here, SnTox3 is used to screen 707 progeny from an eight-founder wheat multi-parent advanced generation inter-cross (MAGIC) population, mapped using 18,640 variable single nucleotide polymorphism (SNP) markers, as well as 198 progeny from the wheat Avalon x Cadenza population, mapped using 8,241 SNP markers. The MAGIC founders, along with Avalon and Cadenza, showed a differing sensitivity to effector SnTox3, with a similar range of sensitivity evident in the progeny. Quantitative trait locus analysis identified a major sensitivity locus on the short arm of chromosome 5B, which was shown to be the *Snn3-B1* locus. Markers linked to *Snn3-B1* identified in these populations can now be converted to the KASP genotyping platform, to provide breeders with simple and cheap diagnostic markers for allelic state at *Snn3-B1*.

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4 – 7 Zane DUXBURY

Understanding and engineering immune receptor complexes in plants.

Zane Duxbury, Jonathan D.G. Jones

The Sainsbury Laboratory, UK

Nucleotide- binding domain and Leucine-rich Repeat-containing (NLR) proteins are the major class of intracellular receptors that confer resistance to pathogens in plants. NLRs recognise and respond to secreted pathogen molecules ("effectors"), which are usually strain-specific. There is relatively little known about the molecular mechanisms that transduce the immune signal from an active NLR to the nucleus to initiate the transcriptional reprogramming required for an effective immune response. The NLR RRS1 contains a WRKY DNA-binding domain and has been hypothesised to directly reprogram the transcriptional machinery during an immune response. Our lab and others have demonstrated that this WRKY DNA-binding domain is acting as bait for pathogen effectors and may not be responsible for direct transcriptional regulation. RRS1 requires both intramolecular and intermolecular interactions to establish an immune signal in cooperation with a partner NLR, called RPS4. I will present recent work that furthers our understanding of the regulation of RRS1/RPS4 activation. This work provides the foundations for understanding how to manipulate RRS1/RPS4 in order to expand recognition specificity. Understanding NLR receptor complexes will provide insight into positive and negative regulation of immune responses and allow us to more effectively transfer disease immunity to disease-susceptible plants.

4 – 8 Valerie GEFFROY

DNA methylation of NB-LRR sequences in common bean genome

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In common bean (*Phaseolus vulgaris*) genome, ~400 NB-LRR (NL) genes have been annotated. Most are organized in complex cluster of genes located in subtelomeric regions close to terminal knobs containing the satellite DNA *khipu*. Phylogenetically related NL genes are spread between different chromosome ends, suggesting frequent exchanges between non homologous chromosomes. NL genes peculiar location, in proximity to heterochromatic regions, led us to study their methylation status using a whole-genome cytosine methylation map at the single-nucleotide resolution. In plant genomes, contrasting patterns of DNA methylation have been identified for transposable elements (TE) and genes: genes are occasionally methylated in CG in their coding region, while TE are usually methylated in the three contexts (CG, CHG, CHH). In common bean, NL genes displayed an unusual body methylation pattern since half of them are methylated in the three different contexts, reminiscent of the methylation pattern of repeated sequences. This was not observed for two other large multigenic families (pentatricopeptide repeat and homeobox transcription factor family) suggesting that this unusual methylation pattern is not ubiquitous to multigenic families. Moreover 90 NL genes were also abundantly targeted by 24nt siRNA, with 90% corresponding to NL genes methylated in the three different contexts. This suggests the existence of a transcriptional gene silencing mechanism of NL through the RdDM (RNA-directed DNA methylation) pathway in common bean which has not been described in other plant species. We are currently studying the dynamic of DNA methylation after pathogen infection.

4 – 9 Anne GIESBERS

Effector-mediated downy mildew resistance discovery in nonhost lettuce accessions

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Classical resistance genes in cultivated lettuce (*Lactuca sativa*) against downy mildew (DM) by *Bremia lactucae* are quickly rendered ineffective by pathogen variability, leading to a devastating yield loss. Consequently, there is a continuous need for novel resistances. Most DM resistance genes in lettuce cultivars have been obtained from the domesticated species (*L. sativa*) itself or from the wild lettuce primary gene pool species *L. serriola.* Few resistance genes have been obtained from the secondary gene pool species. The secondary gene pool species of lettuce include some highly resistant species like *L. virosa and L. georgica,* which are nearly completely resistant, and *L. saligna* that is completely resistant (nonhost species). These three secondary gene pool species comprise a source of potential novel *Bremia* resistance genes that has hardly been exploited. We performed transient expression of 18 previously identified potential RXLR(-like) effector proteins of *B. lactucae* in wild *Lactuca* accessions. Two effector candidates showed a cell-death response. RXLR effector BLR31 is the first identified *B. lactucae* effector that triggers a hypersensitive response in *L. saligna* accessions and cosegregates with complete resistance to the *B. lactucae* isolate from which it originates. Previous genetic studies on the nonhost resistance of *L. saligna* showed involvement of interactive quantitative trait loci (QTLs). Here, we demonstrate that an avirulence effector can distinguish major dominant resistance genes from QTL-based resistance, as well as stacked dominant resistance genes.

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4 – 10 Amelie HECKMANN

Integration of protein kinase in plant NLR receptors: a new way to recognise pathogens

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Plant resistance to pathogen is partially based on the perception of specific pathogen avirulence effectors by plant cytosolic NLR receptors. Recently it has been bioinformatically shown that proteins including plant kinases (PK) have been integrated in some NLRs. We focus our research on predicted NLR-PKs from wheat and barley and test their role in perception of effectors from *Puccinia striiformis f.sp. tritici* (Pst), the damaging pathogen responsible of wheat yellow rust. Using *N. benthamiana* we are conducting *in planta* functional assays to determine the role of integrated kinase. Preliminary co-immunoprecipitation with *Pst* effectors and the kinase part of the NLRs indicate that this integrated domain has a specific binding ability. We are now pursuing those analyses with the full length NLR-PKs. In parallel, we aim to produce in *N. benthamiana* recombinant NLR-PK proteins for the structural biology of this receptor. Insight on this newly discovered type of receptors can provide the insights to other type on integrated domains as well as new possibilities for R gene engineering and resistance introduction in economically important crops.

4 – 11 Matthieu JOOSTEN

SOBIR1 plays a central role in signaling by receptor-like proteins

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Receptor-like proteins (RLPs) involved in disease resistance 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 (Liebrand et al. (2014) TiPS 19:123–132). The RLPs Cf-4 and Cf-9, providing resistance to the fungal tomato pathogen *Cladosporium fulvum*, form a constitutive complex with SOBIR1 (Liebrand et al. (2013) PNAS 110:10010-10015) and recruit SERK3 (BAK1) and SERK1 upon their activation by matching effectors (Postma et al. (2016) New Phytol. DOI:10.1111/nph.13802). SOBIR1 contains a typical "glycine zipper" (GxxxGxxxG) in its trans-membrane domain and we found that this motif is essential for the interaction with RLPs (Bi et al. (2015) Mol. Plant Pathol. 17:96-107), which also contain such a motif. Interestingly, Arabidopsis SOBIR1 shows auto-immunity in tobacco and we observed that this feature is SERK-dependent and also requires the glycine zipper. We found that a bacterial type III-secreted effector targets SOBIR1, thereby inhibiting its auto-immunity. We have generated tyrosine mutants in the kinase domain of SOBIR1 that affect auto-immunity and these will be informative for elucidating whether SOBIR1, in addition to being a binding scaffold for RLPs, is also involved in downstream phosphorylation processes initiating defense signaling. Future studies will focus on (1) the role of the glycine zippers, which are also present in the SERKs, in (tripartite) complex formation; (2) potential differential phosphorylation of SOBIR1 upon activation of the associated RLP by ligand recognition; (3) the precise impact on SOBIR1 function upon its targeting by a bacterial effector and (4) the identification of (cytoplasmic) signaling proteins that are recruited downstream of the Avr4/Cf-4/SOBIR1/SERK complex.

4 – 12 Sung-yong KIM

Editing a net blotch susceptibility gene for disease resistance in barley using CRISPR

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The gene-for-gene resistance concept is applied to cases when a certain plant resistance gene recognizes the pathogen and triggers plant defense mechanisms. This type of resistance is widely used to breed crops for resistance to pathogens. However, there are also other types of monogenic resistance exploited in crop breeding, such as those for loss of susceptibility. Powdery mildew, one of the major fungal diseases of barley in Europe, may be controlled based on natural mutation in a susceptibility gene, called *MLO*. *MLO* is needed for the powdery mildew to enter into host epidermal cells and mutations in *mlo* make the plant resistance to all current powdery mildew races. In wheat, inactivation of *MLO* gene has also been achieved using gene editing technology with the loss of gene function in all three genomes of wheat. The necrotrophic fungal pathogen *Pyrenophora teres* f. *teres* causes the destructive foliar disease net blotch with yield losses of up to 40% in barley. The chromosome 6H centromeric region of barley harbours net blotch resistance and susceptibility loci. Dominantly inherited susceptibility has been found in two barley cultivars, Rika (R) and Kombar (K), in relation to two different isolates of net blotch. This gene is designated *Spt1.R/Spt1.K* (susceptibility to *P. teres* f. *teres*). In my presentation, I will present the latest progress of our study where we try to inactivate the *Spt1* gene in barley using CRISPR/Cas9 for obtaining net blotch resistance. Thus we expect that the corresponding necrotrophic fungal pathogen effectors will not be recognized and these races of the pathogen should thereby no longer be able to manipulate its host.

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4 – 13 Valentina KLYMIUK

Towards positional cloning of a new stripe rust resistance gene *YrG303* derived from wild emmer wheat

Valentina Klymiuk[1], Dina Raats[1], Zeev Frankel[1], Valeria Bocharova[1], Abraham Korol[1] and Tzion Fahima[1]

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Stripe rust, caused by the fungus *Puccinia striiformis* f.s. *tritici* (*Pst*), is one of the most destructive pathogen of wheat globally. Yield losses can be severe due to shriveled grains and damaged tillers and they can reach up to 80% of all yield in susceptible varieties. Depletion of effective resistance to *Pst* in cultivated wheat has led to a search for new genes among wild progenitors. *YrG303*, derived from wild emmer wheat, *Triticum dicoccoides*, is a major dominant gene that we have identified and mapped to wheat chromosome 1BS. The main objective of our project is to clone *YrG303* using the positional cloning approach. Fine mapping of *YrG303* was conducted based on graphical genotyping of a large mapping population developed by crossing the donor *T. dicoccoides* accession G303 with *T. durum* cv D447. A modern technology of KASP markers was used in addition to SSR and CAPS markers for fine mapping of the target gene. We are currently refining *YrG303* physical map by additional genetic markers using the 1BS physical maps and survey sequence of bread wheat "Chinese Spring". The isolation of this new stripe rust resistance gene will enrich the available gene pool of resistance to this destructive pathogen, and will broaden our understanding of plant immunity.

4 – 14 **Marjin KNIP**

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

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Nucleotide-binding Leucine-rich Repeat proteins (NLRs) are a highly conserved, major class of immune receptors. We've developed 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*. 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. 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've developed 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.

4 – 15 **Marc-Henri LEBRUN**

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

Marc-Henri LEBRUN[1], Thierry LANGIN[2], Thomas KROJ[3], James COCKRAM[4], Richard OLIVER[5], Gert KEMA[6], Romain VALADE[7], Sébastian PRAUD[8], Valérie LAURENT[9], Laure DUCHALAIS[10], Benoit FOUCAULT[11]

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Fungal effectors represent an opportunity for breeding crops resistant to diseases. This game-changing technology will create novel opportunities and methods to identify plant resistances. In addition, effector-based screening is amenable to high throughput protocols facilitating detection and introduction of resistance genes in elite cultivars. The objective of WEAB is to use toxic protein effectors from four fungal pathogens of wheat, *Fusarium graminearum (Fg)*, *Zymoseptoria tritici (Zt), Pyrenophora tritici-repentis (Ptr)* and *Parastagonospora nodorum (Pn)* to detect disease resistance genes/QTLs in collections of wheat cultivars. These aims are greatly complemented by the availability of high-density genetic marker coverage of wheat genome, and development of GWA methods. Recombinant protein effectors are produced in yeast and purified proteins are delivered into wheat leaves by syringe infiltration. Symptom development is scored five days after infiltration. Screening of 215 elite European winter wheat cultivars with *Pn* necrotrophic effectors ToxA, 1 and 3, has highlighted a large number of cultivars resistant to the 3 (110 cultivars, 51 %). Only few cultivars (2) are sensitive to these three effectors. This situation is strikingly different from Australian wheat cultivars that are mainly sensitive to these 3 necrotrophic effectors (no cultivar insensitive to these three effectors). This suggests that previous breeding for field resistance to *Pn* in Europe (1960-1980) was successful in pyramiding ToxA, 1 and 3 insensitivity alleles. To validate this hypothesis, these wheat cultivars are currently pathotyped with European *Pn* isolates including a French isolate producing *Pn* Tox1 and 3, in controlled and field conditions. Mapping of loci controlling insensitivity to *Pn* necrotrophic effectors and resistance to *Pn* isolates will be performed using genome-wide association analyses. Candidate effectors from *Z. tritici* and *F. graminearum* are also currently produced as recombinant proteins in yeast to test their possible toxicity on wheat. Identification of candidate wheat effector targets (WET) are also performed using yeast two hybrid screening. WET genes will be localized on wheat genome allowing their mapping relative to known resistance QTLs. This project will facilitate breeding efforts to select for resistance to important fungal pathogens in wheat by providing a 'toolkit' of effector-based molecular markers.

4 – 16 Min LIN

Can sensitivity to necrotrophic effectors explain differences in host resistance to *Parastagonospora nodorum* blotch in European winter wheat?

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The necrotrophic fungus *Parastagonospora nodorum*, the cause of *Stagonospora nodorum* blotch (SNB), is an important pathogen of wheat (*Triticum aestivum* L.), which not only causes yield losses but also reduces grain quality. In contrast to other European countries, *P. nodorum* is the dominant component of the leaf blotch disease complex in Norway. Since commercial wheat varieties lack sufficient SNB resistance, chemical control is still the main method to reduce SNB damage. According to former studies, *P. nodorum* can trigger host cell death by secreting necrotrophic effectors (NEs), which specifically correspond to host sensitive loci, in order to accelerate infection and utilize nutrients from dead host tissues. Mapping quantitative trait loci (QTL) for NE sensitivity loci provides a useful strategy to approach breeding for genetic resistance to SNB. In this study, two eight-parent wheat MAGIC (multiparent advanced generation inter-cross) populations and a Norwegian association mapping panel have been used for QTL mapping SNB resistance. Preliminary results show a high diversity of NEs in the Norwegian *P. nodorum* population. Some of the QTL identified from the field data will be investigated further by seedling experiments in the greenhouse to investigate whether they are based on novel NE sensitivities. Ultimately, these results will be used to develop molecular tools to select against susceptibility loci in breeding materials and facilitate effective breeding for SNB resistance.

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4 – 17 Charlotte NELLIST

Improving disease resistance in strawberry

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The global strawberry industry faces major challenges, with growers encountering increasingly unpredictable and variable weather conditions, as well as the withdrawal of many fungicides and soil fumigants. These challenges are resulting in increased strawberry crop losses due to soil-borne diseases such as strawberry crown rot and strawberry red core, caused by the oomycete pathogens *Phytophthora cactorum* and *Phytophthora fragariae*, respectively. This project aims to identify and characterise pathogen effectors necessary for the infection process, investigating the underlying pathogenicity factors, as well as identify and map resistance quantitative trait loci (QTL) in strawberry (*Fragaria* spp.) and examine the mechanisms of resistance. There has been extensive research investigating qualitative (major gene) resistance to *Phytophthora* species, however, much less is known about quantitative resistance (multiple genes, each of partial effect). Here we present our progress to date in identification of the genetic basis of quantitative resistance to *P. cactorum* in the octoploid strawberry (*Fragaria* x *ananassa*). A mapping population of 'Emily' x 'Fenella' was assessed for resistance/susceptibility to *P. cactorum* and multiple QTL were identified, all of which contain one or more candidate NB-LRR genes within the QTL intervals. Analysis of multiple *P. cactorum* genomes has focused on exploring the repertoire of RxLR and Crinkler effectors. Studying both pathogen and host diversity simultaneously, will enable us to provide more durable resistance against these devastating soil-borne pathogens.

4 – 18 Liliya PYLYPENKO

Identification of sources of resistance to cereal cyst nematode in common wheat cultivars from Ukraine

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Polymorphism of the Ukrainian common wheat cultivars with use of the molecular marker of the *Cre8* gene conferring moderate resistance to cereal cyst nematode (CCN) *Heterodera avenae* was studied. 40 common wheat cultivars bred for different climatic zones in Ukraine were screened with the molecular genetic marker *wri15* (Jayatilake *et al,* 2015) revealing only three cultivars with the resistance allele at the *Cre8* locus*: Mironovskaya 66, Mironovskaya 68* and *Mirlena* (developed at the Remeslo Institute of Wheat, Mironivka). Genealogy investigation showed old German wheat cultivars in these cultivars' pedigree. Since cereal cyst nematode can greatly affect wheat production causing yield loss of susceptible cultivars up to 50%, these newly identified sources of resistance offer an opportunity to improve wheat breeding program in Ukraine targeting on CCN resistance. Further extensive study of wheat germplasm from Ukraine is needed to detect plant material carrying not only *Cre8* but also other CCN resistance genes.

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4 – 19 Yuan QIN

The type-III secretion system (T3SS) of *Pseudomonas syringae* pv. *syringae* 61 (*Psy*61) may be recognized in *Triticum aestivum*

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The type-III secretion system (T3SS) occurs widely in Gram-negative bacteria that are pathogenic on plants and animals. T3SS is a specialized multi-protein pilus, organized to secrete and deliver proteins (called effectors) into the host cytosol, and occasionally into the extracellular environment, to facilitate efficient disease development. Effector-to-Host Analyzer (EtHAn) is an artificially engineered strain of the non-pathogenic soil bacterium, *Pseudomonas fluorescens* Pf0-1, in which the T3SS coding *hrp*/*hrc* region from plant pathogenic *P. syringae* pv. *syringae* 61 is stably integrated. EtHAn is mainly used to analyze type-III effectors, while less is known about plant targets of T3SS itself. Therefore, 440 wheat (*Triticum aestivum*) lines (Wagtail lines) already marker-genotyped were used to screen for genes mediating T3SS recognition. This was done by leaf infiltration of an EtHAn bacterial suspension using a blunt-end syringe. The wheat lines showing a hypersensitive response-like phenotype were recorded, and the response was found to associate with a 2-cM interval in the wheat genome. At the same time, wild-type *P. fluorescens* Pf0-1 was found to have no effect on most of the lines. We are currently aiming to resolve which protein of the T3SS is recognized, and at the same time we are trying to limit the wheat genome interval to find the gene encoding the recognizing protein.

4 – 20 Manon RICHARD

Deciphering the role of NLR immune receptors DNA binding /damage in plant immunity

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Plants have evolved a sophisticated immune system to protect individual cells, and hence safeguard the entire plant, against pathogen infection. Nucleotide-binding leucine-rich repeat (NLR) immune receptors perceive non-self and modified-self molecules inside host cells and are the key actors of resistance success. However, how NLR proteins are able to initiate immune signaling remains largely unknown. The potato NLR protein Rx1 confers resistance to Potato Virus X (PVX) by recognition of its viral coat protein (CP). CP recognition leads to activation of the Rx1 protein, and it has recently been shown that activated Rx is able to bind DNA *in planta* and *in vitro* and to induce DNA damage *in vitro*. The role of this unexpected biochemical activity of NLR-type immune receptors in resistance against pathogens remain to be discovered. This study aims to uncover (i) which sequences are targeted by Rx1 and (ii) which correlation exist between Rx1 DNA binding sequences and the rapid transcriptional reprogramming observed during early defense responses.

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4 – 21 Anja RUUD

SnTox3-*Snn3* as a major determinant of field susceptibility to *Septoria nodorum* leaf blotch in the SHA3/CBRD × Naxos population

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The discovery of necrotrophic effectors (NEs) and corresponding sensitivity *(Snn)* genes in the *Parastagonospora nodorum* – wheat pathosystem introduced the inverse gene-for-gene model and raised new hope for resistance breeding against this pathogen, causing Septoria nodorum leaf and glume blotch (SNB). It is the major leaf blotch pathogen in spring wheat in Norway. Several NE/*Snn* interactions play major roles at the seedling stage. However, the effect of these interactions in the field under natural infection has not been investigated. We saturated the existing SSR map of the recombinant inbred line (RIL) population SHA3/CBRD × Naxos using the Illumina 90K SNP chip. The population had previously been evaluated for SNB susceptibility in field trials. Using SnTox3-sensitivity as a phenotypic marker, we could map the *Snn3* locus on 5BS, 0.1 cM from the most closely linked SNP markers. We also conducted inoculation and culture filtrate (CF) infiltration experiments at the seedling stage with four selected *P. nodorum* isolates from Norway and North America. Re-mapping of QTL for field resistance showed that the SnTox3-*Snn3* interaction could explain more than 24 % of the phenotypic variation in the field, and more than 51 % of the variation in seedling susceptibility in this population. To our knowledge, this is the first time the effect of this interaction has been documented at the adult plant stage under natural infection in the field.

4 – 22 Octavina SUKARTA

Exploring the *Resistosome* of the Potato CC-NB-LRR Immune Receptor Rx1

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The potato Rx1 is an intracellular Nucleotide-binding Leucine Rich Repeat (NLR) immune receptor with an archetypical N-terminal coiled-coil (CC) domain. It confers extreme resistance against Potato Virus X (PVX) by gene-specific recognition of the viral coat protein (CP). Recent findings point to a role of Rx1 in the nucleus whereby it could directly bind host genetic material, though it remains unclear how this process eventually leads to defence. A possibility is that Rx1 recruits other host factors, for example via the CC domain, which is predicted to act as scaffolds for nuclear signalling. Here, we used the CC domains of Rx1 and the Rx1-like protein Gpa2 (mediates defence against the nematode *Globodera pallida*) as baits in a Co-IP/MS analysis after cell fractionation 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 confirmed complex formation with the full-length immune receptors *in plantae*. Interestingly, co-expression of Rp05 alters the subcellular distribution of the Rx1-CC domain, hinting its role in Rx1-function. Transient overexpression experiments confirm that Rp05 could in fact potentiate defense against PVX. Interestingly, however, this occurs independently of Rx1. We substantiated this model by demonstrating that Rp05 could influence HR-responses by other NLR proteins (e.g. Gpa2, Sw5A/B and Mi-1) indicating that it may be a common downstream component in immune signaling. Currently, we focus on elucidating the detailed molecular underpinning of Rp05 function in R-gene mediated resistances using Rx1 as the principal model system.

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4 – 23 Michel VAN THOURNOUT

The impact of different sequencing technologies on R-gene cloning strategies

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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. Breeding for disease resistance in new cultivars makes use of endogenous R-genes and marker assisted selection. R-gene cloning and sequencing approaches enable the development of these molecular markers. However, regions harboring disease resistance genes are highly variable and prone to tandem and segmental duplication, leading to highly repetitive sequence stretches. Regions containing gene duplications with high sequence similarity have led to miss-assemblies when using short-read sequencing technologies, thus impeding their use in the context of disease resistance genes. Here, we present the use of long read sequencing (PacBio, Oxford Nanopore) to identify genes in complex repeat regions. We show that the assembly of a BAC based on long reads has successfully identified two copies of putative Clubroot disease resistance genes lying in tandem, which could not be resolved in the short read assembly.

4 – 24 Andrea Paola ZULUAGA

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

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Identifying new sources of disease resistance and new resistance mechanisms is still a challenge, in particular in Monocots. Moreover, most biological disease resistance pathways identified so far are not efficient under abiotic stress. This is largely due to negative cross-talks between disease resistance and abiotic tolerance. We have demonstrated the role of one protein containing BED domains, called ZBED, in disease resistance in rice. Quite interestingly, ZBED over-expressor plants also show increased drought tolerance in the field. Thus, this gene represents one of the very few cases where disease resistance and drought tolerance are simultaneously improved. This suggests that ZBED is controlling different biological pathways than those known in disease resistance and drought tolerance. The aim of this work is to understand the molecular function of the ZBED protein and the mechanisms it controls to confer disease resistance. Here we show the preliminary results to understand the role of BED proteins and the novel mechanism of plant immunity and its interaction with drought and salinity tolerance.

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