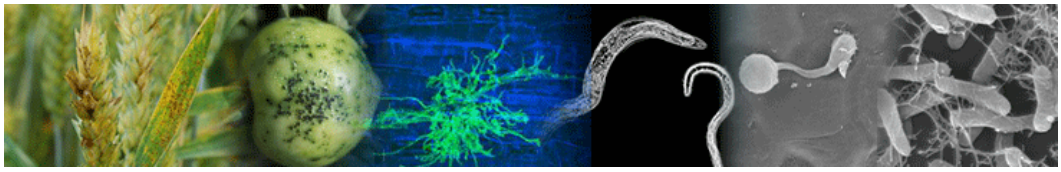
3rd Annual Conference of the COST Action Sustain (FA1208)

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

PROGRAM & ABSTRACT BOOK



INRA SCIENCE & IMPACT

SFP Société Française de Phytopathologie

LANGUEDOC ROUSSILLON LA RÉGION MIDI PYRÉNÉES

cost EUROPEAN COOPERATION IN SCIENCE AND TECHNOLOGY

MEETING OVERVIEW

Wednesday 17th February	Thursday 18th February	Friday 19th February
8:30 - 8:45 Welcome		
8:45 - 10:30 Session 1 Effectors and Virulence	**8:45 - 10:30** Session 3 *R* Genes and Effector-Informed Resistance	**8:45 - 10:30** Session 4 Effector Targets
Break	**Break**	**Break**
11:00 - 12:20 Session 1 Effectors and Virulence	**11:00 - 12:20** Session 3 *R* Genes and Effector-Informed Resistance	**11:00 - 12:20** Session 4 Effector Targets **12:20 - 12:30** Concluding Remarks
12:30 - 14:00 Lunch	**12:30 - 14:00** Lunch	**12:45-14:00** Lunch
14:00 -15:25 Session 2 Evolution & Diversification	**14:00 - 14:30** Presentation of Activities of the Sustain Action & Plenary Discussion	
Break	**14:30 - 15:30** Management Committee Meeting	
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20:00 Dinner	**20:00** Dinner	

FA1208

Pathogen-informed strategies for sustainable broad-spectrum crop resistance

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

Organizing Committee

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How plant pathogenic bacteria manipulate the plant

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

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

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

10:30-11:00 BREAK

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

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

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

12:00-12:20 **MICKAEL QUENTIN** *(INRA, Sophia- Antipolis, FR)*
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5	Janos	BINDICS	Identification of *Ustilago maydis* Effectors Targeting Hormonal Signaling
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9	James	COCKRAM	EffectaWheat : An Effector- and Genomics-Assisted Pipeline for *Necrotrophic Pathogen* Resistance Breeding in Wheat
10	Anna	COLL	StERF, an Ethylene Response Factor involved in potato defence response to PVY
11	Karine	DE GUILLEN	*Magnaporthe oryzae* effectors AVR-Pia and AVR1-CO39 Reveal Structural Homology
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ABSTRACTS OF ORAL PRESENTATIONS

Wednesday 17th of February 2016

Session 1

PATHOGEN EFFECTORS AND VIRULENCE

Ulla BONAS

How plant pathogenic bacteria manipulate the plant

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

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

Nico TINTOR

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

Nico Tintor[1], Peter van Dam[1], Libera Lo Presti[2], Regine Kahmann[2] and Martijn Rep[1]

1 Molecular Plant Pathology, University of Amsterdam, the Netherlands
2 Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

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

Alice MOREL

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

Alice Morel[1], Patrick Barberis[1, 2], Xavier Barlet[1, 2], Gaofei Jiang[1, 2], Fabien Lonjon[1, 2], Fabienne Vailleau[1, 2,3], Stéphane Genin[1, 2] and Nemo Peeters[1, 2]

1 INRA, Laboratoire des Interactions Plantes Micro-organismes (LIPM), UMR441, Castanet-Tolosan, France
2 CNRS, Laboratoire des Interactions Plantes Micro-organismes (LIPM), UMR2594, Castanet-Tolosan, France
3 Université de Toulouse; INP; ENSAT; 18 chemin de Borde Rouge, 31326 Castanet-Tolosan, France

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

Salim BOURRAS

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

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

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2 Current address: Institute of Plant Sciences, Agricultural Research Organization, Volcani Center, 50250 Bet Dagan, Israel
3 Current address: KWS Saat AG, Grimsehlstrasse 31, 37555 Einbeck, Germany
4 Institute of Evolution, University of Haifa, Mount Carmel, 31905 Haifa, Israel

In cereals, several mildew resistance genes occur as large allelic series. In wheat, 17 functional Pm3 alleles confer agronomically important race-specific resistance. While the molecular basis of race-specificity is well characterized in wheat, little is known about allele-specific avirulence in powdery mildew since no avirulence gene for Pm3 was cloned. Here, we dissected the genetic network of virulence/avirulence towards six Pm3 alleles and found that it is controlled by three major Avr loci, with a common locus_1, encoding for a candidate effector gene, and involved in all AvrPm3-Pm3 interactions. We cloned the effector gene AvrPm3a2/f2 from locus_2 which is specifically recognized by the Pm3a and Pm3f alleles. Specificity was demonstrated by induction of an allele-specific hypersensitive response in transient assays in Nicotiana benthamiana and in wheat. Gene expression analysis of locus_1 encoded effector gene Bcg1 and AvrPm3 a2/f2 revealed significant quantitative differences between isolates, indicating that in addition to protein polymorphisms, expression levels play a role in virulence/avirulence. We propose the “Avr-R-Svr” model involving three components for race-specificity: an allele-specific avirulence effector (Avr), a resistance gene allele (R), and a pathogen-encoded suppressor of avirulence (Svr). Thus, whereas specificity is controlled by a genetically simple allelic series in wheat, recognition and suppression of recognition on the pathogen side is more complex, allowing flexible evolutionary responses and adaptation to resistance genes.

Hesham GIBRIEL

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

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

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

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

Diana NAALDEN

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

Diana Naalden[1], Annelies Haegeman[2], Silke Nowak[1], Nguyen Xuan Huy[1], Romnick Latina[1], Godelieve Gheysen[1]

1 Department of Molecular Biotechnology, Ghent University, Coupure links 653, 9000 Ghent, Belgium
2 Plant Sciences Unit, Institute for Agricultural and Fisheries Research (ILVO), Caritasstraat 21, B-9090 Melle, Belgium

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

Suayib USTUN

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

Suayib Üstün[1], Arsheed Sheikh[3], Alex Jones[3], Wolfgang Hoehenwarter[4], Vardis Ntoukakis[3] and Frederik Börnke[1, 2]

1 Leibniz-Institute for Vegetable and Ornamental Crops (IGZ), Großbeeren, Germany
2 Institute for Biochemistry and Biology, University of Potsdam, Germany
3 School of Life Sciences University of Warwick, Coventry, United Kingdom
4 Leibniz Institute for Plant Biochemistry, Halle (Saale), Germany

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

Mickael QUENTIN

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

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

Session 2

EFFECTOR EVOLUTION AND DIVERSIFICATION

Francis MARTIN

Unearthing the Roots of *Fungal mycorrhiza*l Symbioses

Francis M. Martin[1], Annegret Kohler1, Alan Kuo[2], László G Nagy[3], Emmanuelle Morin[1], Igor V Grigoriev[2], David Hibbett[3] & Mycorrhizal Genomics Initiative (MGI) Consortiumx

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

Eva STUKENBROCK

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

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

Déborah MERDA

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

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

Charlotte VAN DER DOES

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

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

Maik RESCHKE

Insights into the evolution of *Xanthomonas* TAL-Effectors

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

Daniel Croll

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

Fanny Hartmann, Andrea Sánchez Vallet, Daniel Croll

Institute of Integrative Biology, ETH Zurich, Switzerland

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

Jean-Benoit MOREL

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

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

Thursday 18th of February 2016

Session 3

R GENES & HOST TARGETS FOR RESITANCE BREEDING AND ENGINEERING

Gregory MARTIN

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

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

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

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

Matthieu JOOSTEN

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

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

Abbas MAQBOOL

Structural basis of effector recognition by a rice NLR immune receptor

Abbas Maqbool[1], Hiromasa Saitoh[2], Marina Franceschetti[1], Clare Stevenson[1], Aiko Uemura[2], Hiroyuki Kanzaki[2], Sophien Kamoun[3], Ryohei Terauchi[2], Mark Banfield[1]

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

Xiao LIN

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

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

Frank TAKKEN

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

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Fusarium oxysporum f. sp. lycopersici (Fol) is the causal agent of tomato wilt disease. During infection the fungus secretes at least 50 proteins - including 30 enzymes and 14 candidate effectors - into the xylem sap. For the Six8 effector we found a potential host target; pull-down assays identified a member of the TOPLESS (TPL) family. The interaction of SIX8 with TPL and with a TPL homolog (TPR1-Topless Related 1) was confirmed in Y2H and BifC experiments. Silencing TPL/TPR1 in tomato resulted in increased resistance to Fol, classifying TPLs as as genuine susceptibility genes.TPLs are co-repressors interacting with transcription factors that are involved in development, hormone signalling, and in “SNC1-mediated” defence mechanisms1. SNC1 is a NB-LRR resistance protein, providing a direct link between Six8 and host defence signalling. Transgenic Arabidopsis plants expressing SIX8 exhibit a temperature-dependent dwarf phenotype and show constitutive defence gene (PR1 and PR2) expression, suggesting a direct link to SNC1-mediated defences.Arabidopsis T-DNA insertion lines lacking SNC1 or components of the SNC1-defence pathway (i.e. tpl, tpr1, tpr3, eds1, pad4, and NahG) have been transformed with SIX8 to identify the pathways affected by Six8. Bioassays on SIX8-containingArabidopsis plants using P. syringae and H. parasitica revealed that NB-LRR resistance proteins other than SNC1 are unaffected. This study identifies TPL as a genuine Six8 effector target. A possible mechanism of how Six8 triggers SNC1-mediated immune signalling will be presented, providing new leads to use effector-targets for disease resistance.

Ralf KOEBNIK

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

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

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

Ksenia KRASILEVA

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

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

Aska GOVERSE

The *Rx1_'Gpa2* locus in potato: a molecular and genetic framework for engineering novel *NLR* genes

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

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

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

Friday 19th of February 2016

Session 4

PLANT PROTEINS AND PROCESSES TARGETED BY EFFECTORS

Regine KAHMANN

The secreted effector repertoire of smut fungi

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

Sophie MANTELIN

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

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

Laurent NOËL

AN ADAPTOR KINASE CONFERS EXPANDED RECOGNITION SPECIFICITY TO A PLANT NLR

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

Renier VAN DER HOORN

Manipulation of Apoplastic Hydrolases by *Pseudomonas syringae*

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

Maëlle JAOUANNET

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

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Aphids, as other biotrophes, are able to supress plant defences through the secretion of effectors3-6. Aphid effectors are produced in salivary glands and secreted with saliva into the plant tissues during probing and feeding3-7. Sets of predicted putative effectors have been recently identified8,9but only a limited number of these candidate have been characterized to date and have been implicated in promoting/decreasing virulence and activating/suppressing defences10.MpSecA has been identified in Myzus persicae saliva. According to my preliminary results, this effector plays a key role in the infestation success. We have showed that the silencing of the gene encoding MpSecA decreased the ability of M. persicae to infest Arabidopsis. Conversely, plants over-producing MpSecA are more susceptible to aphid infestation. We have recently demonstrated that MpSecA is interacting with two histone H3 arginine methyltransferases, AtPRMTA and AtPRMTB. We didn't observe any significant reduction of M. persicae fitness on single AtprmtA/AtprmtB knockout mutants. Interestingly, a double mutant AtprmtA-AtprmtB appears more resistant to aphid infestation suggesting that both MpSecA and its plant targets are essential to the aphid infestation success.Our fresh results support the hypothesis that aphid MpSecA promotes the host arginine-methyltransferase activity on histone H3 inducing the repression of plant gene expression. Indeed, I have shown that MpSecA induces chromatin over-compaction (Jaouannet and Bos, preliminary data). The functional characterisation of MpSecA are opening up an exciting area of research addresses that chromatin remodelling and/or histone post-translational modifications could act as important regulators of plant defences and thus facilitate the success of aphid infestation.

Yasin DAGDAS

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

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

Nuria SANCHEZ-COLL

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

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

Jan SCHIRAWSKI

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

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

ABSTRACTS

OF POSTER PRESENTATIONS

P1 : Carolina AGUILERA-GALVEZ

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

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

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

P2 : Philip ALBERS

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

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

P3 : Nuno Felipe ALMEIDA

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

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

P4 : Lander BAUTERS

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

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

P5 : Janos BINDICS

Identification of *Ustilago maydis* Effectors Targeting Hormonal Signaling

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

P6 : Eran BOSIS

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

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

P7 : Klaas BOUWMEESTER

Lectin receptor kinases in microbial recognition and plant immunity

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

P8: Maria Angeles CASTILLEJO

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

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

P9: James COCKRAM

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

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

P10: Anna COLL

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

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

P11: Karine DE GUILLEN

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

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

P12: Amalia DIAZ GRANADOS

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

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

P13: Rowena DOWNIE

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

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

P14: Abdelnaser ELASHRY

Investigation of *Heterodera schachtii* transcriptome to identify putative effectors

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

P15: Maria Raffaella ERCOLANO

Comparative genomic approaches for investigating *Solanaceae* defence system

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

P16: Lennart ESCHEN LIPPOLD

Bacterial effector-mediated suppression of PAMP-induced defence signaling

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

P17: Sebastian EVES-VAN DEN AKKER

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

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

P18: Bruno FAVERY

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

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

P19: Sara FONDEVILLA

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

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

P20: Sharon GARRIDO

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

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How plant pathogens adapt to a specific host remains one of the important questions in the field of phytopathology and ecological genetics. One way to understand the underlying molecular mechanism of host specialization and pathogenicity is through the identification and functional analysis of candidate determinants of virulence in a given specific host. We took a transcriptomic approach to identify genes specifically upregulated in a compatible host-pathogen interaction versus a non-compatible interaction of the hemibiotrophic fungus Zymoseptoria tritici. Z. tritici is specialized to infect wheat Triticum aestivum while it cannot establish infectious hyphae in the mesophyll of the grass species Brachypodium distachyon. Based on the RNAseq data at an early and critical stage of infection (4 d.p.i.) on wheat and B. distachyon, we identified four genes (Zt69330, Zt42222, Zt41440 and Zt107320) that were significantly differentially expressed between the two hosts suggesting a determining role in host compatibility. These putative determinant genes have putative functions related to pathogenicity. Zt69330 encodes a glycoside hydrolase; Zt42222, a multicopper oxidase; Zt41440 and Zt107320 are both Zn(II)2Cys6 transcription factors. Using a reverse genetic approach, we analyze the functional roles of the four genes in the Z. tritici-wheat interaction. Plant infection assays of the mutant strain ΔZt69330 reveal a reduced degree of necrosis formation in the mutant supporting a role of Zt69330 in host cell wall degradation and necrotrophic establishment of Z. tritici in wheat. The finding of virulence related genes from the RNAseq datasets from T. aestivum and B. distachyon underlines the power of comparative transcriptome profiling in the search of virulence determinants in pathogens.

P21: Valerie GEFFROY

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

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

P22: Laurence GODIARD

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

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

P23: Rafal HOSER

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

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HopQ (for Hrp outer protein Q1) is a type three effector secreted by many strains of Pseudomonas syringae, a gram-negative bacterium that infects a wide range of plant species. We have previously shown that after delivery into plant cells, HopQ1 is phosphorylated and binds to host 14-3-3 proteins. This interaction affects stability, subcellular localization and possibly bridges HopQ1 to its bona fide virulence target. There is increasing evidence that activation of mitogen-activated protein kinases (MAPKs) is affected by HopQ1. We have observed that indeed, HopQ1 interferes with MAPKs immune signaling in Arabidopsis. Furthermore we have noticed that two strain-specific variants of HopQ1, which differ only at a few amino acid positions, show considerable differences in their ability to suppress MAPKs activities. Moreover, the HopQ1 variants are diversely susceptible to proteolytic cleavage by an unknown host protease, which might regulate function of the effector in plant cells. These data encouraged us to test over 150 P. syringae strains pathogenic to various stone fruit tree species, to find amino acids involved in strain-specific functions of HopQ1. We have observed, that HopQ1 variation correlates with the host range of individual P. syringae strains. It is proposed that the differences between HopQ1 homologs are a consequence of an adaptation of the effector to given hosts of bacterial strains. We are currently testing this hypothesis.This work was supported by National Science Centre Poland (grant number: 2013/11/B/NZ9/01970).

P24: Paolo IOVIENO

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

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

P25: Marjin KNIP

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

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Nucleotide-binding Leucine-rich Repeat proteins (NLRs) are a highly conserved, major class of immune receptors. We're developing a system that allows studying the newly discovered ability of NLRs to bind and nick DNA in planta in relation to the onset of immune responses. NLR-type immune receptors are present in both the nucleus and the cytosol and require both localizations to be fully functional. Very little is known about the molecules targeted by NLR's in immunity. In collaboration with the Cann laboratory, our group has recently found that NLRs can bind and nick DNA in vitro1 . It was also found that DNA binding occurs in vivo, and relies on the activation of the NLR receptor following recognition of its genuine effector1. These findings could provide an explanation why NLR-receptors require a nuclear localization to function: Activated NLRs might trigger immune responses by directly binding and/or nicking DNA. To study the nuclear function of NLR proteins, we're developing a system that allows synchronized induction of NLR-triggered immune responses, by using N. benthamiana plants that constitutively express the potato NLR-protein Rx1 in combination with controlled expression of the PVX-coat protein. This system allows us to study the occurrence, localization and timing of NLR-induced DNA-damage using TUNEL and COMET assays and to monitor the onset of subsequent specific immune responses by, among other methods, qPCR.

P26: Jakub KWIATKOWSKI

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

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HopQ1 (for Hrp outer protein Q), a TTSS effector secreted by Pseudomonas syringae promotes virulence of bacteria in many plant species. Mechanisms underlying HopQ1 function remain largely elusive, however, we have recently determined a few factors, that seem to regulate HopQ1 activity in plant cells. First, after delivery into plant cells HopQ1 is phosphorylated and binds host 14-3-3 proteins, which stabilize the effector, affect its subcellular localization and possibly facilitate its interaction with other host proteins. Furthermore, redox conditions and calcium ion level appear to play an important role in the control of HopQ1 oligomerization and subcellular localization. Size exclusion chromatography coupled to MALS (Multi-Angle Light Scattering) revealed that HopQ1 forms in vitro monomers, dimers and trimers. However, treatment with a reducing agent or mutations in either one of both HopQ1 cysteines abolishes oligomerization, indicating that HopQ1 oligomeric state depends on disulfide bridge formations. On the contrary, under calcium depleted conditions, HopQ1 monomers are reversibly converted to dimers. Similarly mutations within the predicted calcium binding motif (HopQ1-D107A_D108A) produced HopQ1 dimers in vitro and in planta. Our data indicate that dimer assembly promotes nuclear localization of HopQ1. Preliminary experiments show that binding of calcium ions in vitro, results in an increased melting temperature of HopQ1. Collectively, these results suggest that calcium may affect localization and stability of the effector. We hypothesize that calcium ions might be also involved in regulation of HopQ1 interaction with specific targets in plant cells. The studies that address this problem are now under way.

P27: Marc-Henri LEBRUN

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

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

P28: Jana LIBANTOVA

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

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

P29: Fabien LONJON

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

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

P30: Diego LÓPEZ MÁRQUEZ

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

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

P31: Takaki MAEKAWA

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

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

P32: Johana C MISAS VILLAMIL

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

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

P33: Hélène MISSONNIER

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

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

P34: Diana ORTIZ

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

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

P35: Javier PALMA GUERRERO

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

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

P36: Nemo PEETERS

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

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

P37: Hélène PIDON

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

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

P38: Marc PLANAS

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

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

P39: Loris PRATX

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

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

P40: Dov PRUSKY

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

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

P41: Liliya PYLYPENKO

DEVELOPMENT AND VALIDATION OF THE MARKER FOR THE NPR1-LIKE FUSARIUM HEAD BLIGHT RESISTANCE GENE

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272 cultivars of common wheat (Triticum aestivum L.) of Ukrainian breeding were studied using the intron-localized molecular marker INDEL1 of the NPR1-like TDF_076_2D gene associated with moderate type II resistance to Fusarium head blight. It was found out that 61.7% of winter wheat cultivars and 83.3% – of spring wheat cultivars carried the resistant allele. The allelic states of the marker are not specific for different resistance associated alleles of the gene and it cannot distinguish polymorphic cultivars from the susceptible ones. Using the sequences of the alleles of the TDF_076_2D and TDF_076_2A genes (Diethelm et al., 2014) the primers complementary to the different allelic states of the TDF_076_2D gene were developed. As the result of the multiplex PCR 914 bp fragments were obtained in case of the resistant allele peculiar for the genotype SVP72017, 323 bp fragments – in case of the susceptible allele and no fragments – in case of resistant allele peculiar for the wheat cultivar 'Capo'. The marker was validated by analyzing 60 cultivars and the results did not contradict the ones obtained with INDEL1.

P42: Dina RAATS

Rapid isolation of new stripe rust resistance variants in cultivated wheat

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Yellow rust caused by the fungus Pucciniastriiformis f. sp. tritici (Pst) is one of the most destructive diseases of wheat, resulting in yield losses of 10–70 % in susceptible varieties and total losses of harvestable grain during severe epidemics1. Recently emerged new Pst races that have expanded virulence profiles and are adapted to warmer temperatures compared to previous races are threatening over 30% of global wheat yield2. There is an urgent need to identify new sources of resistance against highly virulent races of stripe rust while lessens our dependence on fungicides. Here we propose a novel forward genetics approach to rapidly identify the genetic variants underlying the stripe rust resistance in Ethyl methanesulfonate mutagenized population of elite wheat cultivar Kronos3. Eleven mutant lines with confirmed stripe rust resistance derived from that population were backcrossed to the parental wild type. F2 populations are available for gene isolation. We will perform exome sequencing of F2 phenotypic bulks using a custom (86 Mb) wheat exome capture design developed by Krasileva et al (in preparation). Based on the frequencies analysis of SNP markers mapped to the best-ordered wheat reference genome data the chromosomal regions contain the mutations underling the gain of resistance phenotypes will be identified. In the case the causative mutation will be missing from our initial analyses, we will further investigate the region containing the gene of interest. The gene isolation approach developed here will be applicable to any trait of interest.

P43: Amey REDKAR

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

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

P44: Javier RUIZ ALBERT

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

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

P45: Andrea SANCHEZ VALLET

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

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

P46: Guido SESSA

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

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

P47: Alan SHULMAN

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

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

P48 Waldemar SKOWRON

Tomato R-genes are targeted by miRNA during nematode pathogenesis

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Plant parasitic nematodes (PPN) evoke dramatic changes in developmental program and metabolism of selected root cells. The transcriptome dynamics during PPN infection also reflects an active suppression of defense responses. This is valid both for root knot nematodes and cyst nematodes what was shown in our previous screening of tomato genes affected during potato cyst nematode (PCN) parasitism and other studies. Among genes downregulated by nematode effectors there are classical NB-LRR R-genes, WRKY regulators and other stress related sequences. Since downregulation of tomato genes on PCN infection accounts for >30% of the total transcriptome changes, we hypothesize that miRNA mediated cleavage may play here an important role. Therefore using the microRNA-seq data we monitored the changes in micro-transcriptome with respect to defense related targets. The NRCH (Solyc04g007050) is one of previously identified PCN suppressed tomato R-genes. In this research we demonstrate that NRCH is a potential target of miR159 and miR9474 present in roots. The downregulation of NRCH gene, which was documented by qRT-PCR, coincides with higher level of these miRNAs in infected roots. Such correlation was discovered in a few other cases. Moreover the microRNA-seq revealed that as much as 67% (149/221) of tomato R-genes are potentially targeted by root miRNAs which are identical or highly homologous to miRBase records (“known” miRNAs). The verification by qRT-PCR of nematode-regulated miRNAs and potential R-gene targets showed reverse correlation in the case of 14 miRNA/target transcript pairs. Mapping of cleavage sites with RACE confirmed some of postulated miRNA activities. In addition to presented data it was shown that only less than 10% of the tomato root micro-transcriptome can be defined as “known” indicating the existence of huge unexplored array of potential miRNA/R-gene interactions.

P49: Yin SONG

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

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

P50: Jana STREUBEL

Dissection of TALE-mediated transcriptional enhancement

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Plant pathogenic Xanthomonas bacteria translocate transcription activator-like effectors (TALEs) into host cells to specifically induce transcription of target genes. The direct binding of TALE proteins to promoter sequences is mediated by a highly conserved region that is composed of tandem 34-amino acid-repeats. These repeats mainly vary in two amino acids called repeat-variable diresidue (RVD) in which one amino acid specifically recognizes one base in the target DNA sequence1. This simple and modular architecture allows the rearrangement of repeats to generate artificial TALEs with any desired DNA-binding specificity and thereby targeted gene regulation. Although the DNA-binding mode is almost fully understood it is still ambiguous how TALE proteins activate transcription and whether additional promoter elements are needed to support efficient gene activation. Natural TALE proteins preferentially bind to core promoter regions ranging from -300 to +200bp around the transcription start site2. By using the example of the OsSWEET14 promoter we systematically positioned TALEs in proximal as well as distal promoter positions and compared their activity. Interestingly, we found that TALEs mediate robust gene activation based on diverse promoter positions although their efficiency varies. Moreover, we showed that TALEs can activate transcription by binding to the reverse strand of the OsSWEET14 promoter, which has not been described, before. Most, but not all of the analyzed TALEs shift the transcriptional start site at the OsSWEET14 promoter demonstrating a differentiated influence on transcriptional initiation. In summary, the highly position- and orientation-independent activation potential suggests that TALEs function similar to enhancer-binding proteins.

P51: Octavina SUKARTA

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

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

P52: Magdalena SWIECICKA

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

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

P53: Boris SZUREK

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

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

P54: Suayib USTUN

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

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

P55: Fabienne VAILLEAU

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

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

P56: Aranka VAN DER BURGH

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

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

P57: Michel VAN THOURNOUT

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

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

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