COST FA 1208

Pathogen-informed strategies for sustainable broad-spectrum crop resistance

1st Annual Meeting

9th – 11th October 2013, Birnam

Meeting overview

Wednesday 9th October	Thursday 10th October	Friday 11th October
08:30 – 10.15 Welcome & WG1 presentations	08.30 – 10.00 WG3 presentations	08.30 – 10.00 WG4 presentations
	Coffee Break	
10.45 -12.15 WG1 presentations	10.30 – 12.15 WG3 presentations	10.30-12.00 WG4 presentations
	Lunch	
13.45 – 15.15 WG2 presentations	13.45-15.00 WG planning	Depart
Break	15.30-17.00 MC meeting	
15.45 – 17.55 WG 2 presentations	15.30-18.30 Posters (Birnam House Hotel)	
Evening meal **Birnam House hotel** **19.30**	**Buffet meal** **Drinks reception** **Live music** **Birnam Institute** **19.00**	

Wednesday, October 9

8:30-8:45 **Welcome and Introductory Remarks**

Thomas Kroj, Chair of COST Action FA1208 and John Jones, Local Organizer

Pathogen effectors and virulence (WG1) –

Chairs: John Jones and Bart Thomma

8:45-9:15 **Mantelin, S.[1], Thorpe, P.[1,2], Cock, P.J.[1], Coke, M. [2], Urwin, P.E.[2] & Jones, J.T.[1] (James Hutton Institute & University of Leeds)**

Identification and functional characterisation of *G. pallida* effectors and their host targets.

9:15-9:35 **Mathieu Gourges (INRA/ University of Nice Sophia-Antipolis, FR)**

Two penetration specific effectors from *Phytophthora parasitica* modulate hormonal physiology of the host to facilitate infection.

9:35-9:55 **Ali Ahmed, Carsten Pedersen & Hans Thordal-Christensen (Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Denmark)**

Functional analysis of barley powdery mildew effector candidates and identification of their barley targets.

9:55-10:15 **Hazel McLellan, Petra C. Boevink, Leighton Pritchard, Miles R. Armstrong and Paul R.J. Birch (University of Dundee/ James Hutton Institute, UK)**

An RxLR effector from *Phythophthora infestans* prevents nuclear accumulation of two potato NAC transcription factors

10:15-10:45 Break

10:45-11:15 **Amey Redkar, Lena Schilling, Alexandra Matei, Christoph Hemetsberger, Ziba Ajami-Rashidi, Virginia Walbot & Gunther Doehlemann (Max-Planck-**

Institute for Terrestrial Microbiology, Germany)

Formation of plant tumors in the *Ustilago maydis* – maize interaction requires organ-specific activity of secreted effector proteins.

11:15-11:35 **Stam R., Motion, G.B., Boevink, P.C. & Huitema, E. (University of Dundee/ James Hutton Institute, UK)**

A *Phytophthora capsici* CRN effector targets a tomato TCP transcription factor to affect its function.

11:35-11:55 **Kombrink, A., Sánchez-Vallet, A., Faino, L., Valkenburg, D.J., van den Berg, G. & Thomma, B.P.H.J.(Wageningen University, NL)**

Suppression of Chitin-Triggered Immunity, and Other Functions of Fungal LysM Effectors.

11:55-12:15 **G. Gheysen, L. Bauters, S. Nowak, D. Naalden & A. Haegeman (Ghent University)**

Effector analysis of two different types of rice nematodes, the sedentary *Meloidogyne graminicola* and the migratory *Hirschmanniella oryzae*.

12:15-13:45 Lunch

Plant proteins and processes targeted by effectors (WG2)

Chairs: Hans Thordal-Christensen and Nemo Peeters

13:45-14:15 **Dionne Turnbull, Susan Breen, Miles Armstrong, Stefan Engelhardt, Licida Giuliani, Ingo Hein, Paul Birch & Eleanor Gilroy (University of Dundee & James Hutton Institute, UK)**

Pumped up on (brassino)steroids: the late blight pathogen manipulates the BR pathway in potato.

14:15-14:35 **Angela Chaparro-Garcia, Tolga Bozkurt, Sebastian Schornack & Sophien Kamoun (The Sainsbury Laboratory, UK)**

Phytophthora infestans RXLR effector AVR3a targets a GTPase involved in plant immunity.

14:35-14:55 **Du, Y. Bouwmeester, K. & Govers, F (University of Wageningen, NL)**

A novel host target of *Phytophthora infestans* RXLR effector.

14:55-15:15 Giska, F., Taube, M., Piechocki, M., Hoser, R., Kozak, M., Hennig, J. & Krzymowska, M. (Institute of Biochemistry and Biophysics, PL)

Interactions of HopQ1, a type III secretion effector from *Pseudomonas syringae*, with host 14-3-3 proteins.

15:15-15:45 Break

15:45-16:15 Mark Kwaaitaal, Mads Eggert Nielsen & Hans Thordal-Christensen (University of Copenhagen, DK)

Analysis of barley cell vesicle trafficking after powdery mildew invasion reveals the identity of the extrahaustorial membrane

16:15-16:35 Jose L. Lozano-Torres, Ruud H.P. Wilbers, Sonja van Warmerdam, Anna Finkers-Tomczak, Casper C. van Schaik, Hein Overmars, Jaap Bakker, Aska Goverse, Arjen Schots & Geert Smant (University of Wageningen, NL)

Secreted venom allergen-like proteins of plant-parasitic nematodes modulate defence responses in host plants.

16:55-17:15 Streubel, J., Pesce, C., Hutin, M., Koebnik, R., Boch, J. & Szurek, B (University of Halle, IRD Montpellier & U. Louvaine)

Rice SWEETs as virulence targets for *Xanthomonas* TALEs.

17:15-17:35 Teper, D., Salomon, D., Sunitha, S., Mudgett, M.B. & Sessa, G (Tel Aviv University, IL & Stanford University, USA)

Xanthomonas campestris type III effector XopQ interacts with tomato and pepper 14-3-3 isoforms to suppress effector-triggered immunity.

17:35-17:55 Tolga O. Bozkurt, Khaoula Belhaj Angela Chaparro-Garcia & Sophien Kamoun (The Sainsbury Laboratory, UK)

An endocytotic pathway targeting the vacuole is re-routed towards pathogen penetration sites in Plants.

19.30: Dinner at Birnam House Hotel

Thursday, October 10

Effector evolution and diversification (WG3)

Chairs: Eva Stukenbrock and Didier Tharreau

8:30-9:00 **Thierry Rouxel (INRA-Grignon, FR)**

Evolutionary dynamics of avirulence genes in *Leptosphaeria maculans*.

9:00-9:20 **Cano, L.M.1, Cooke, D.E.L.2, Raffaele, S.3, Pais, M.1, Oliva, R.F.4, Etherington, G.1, Birch, P.R.J.2, Coffey, M.5 & Kamoun, S. (The Sainsbury Laboratory, James Hutton Institute, University of Dundee, IRRI & U Tolosan)**

Genome sequencing and expression profiling of emerging strains of *P. infestans.*

9:20-9:40 **Helder, Johannes, Van den Elsen, Sven, Mooijman, Paul, Kasia Rybarczyk, Landeweert, Renske & Goverse, Aska (Wageningen Universit, NL)**

Insights into the evolution of plant parasitism within the phylum Nematoda based on neutral and pathogenicity-related genes.

9:40-10:00 **Fournier E, Ortega-Abboud E, Mallet L, Chiapello H., Guérin C., Rodolphe F., Gendrault A., Kreplak J., Amselem J., Philippe N., Lebrun M-H., Kroj T. & Tharreau D. (INRA/CIRAD-Montpellier, FR)**

Evolution of the pan-secretome among lineages of *Magnaporthe oryzae* attacking different host-plants.

10:00-10:30 Break

10:30-11:00: **de Jonge, R., Bolton, M.D., Kombrink, A., van den Berg, G.C., Yadeta, K.A. & Thomma, B.P.H.J (Wageningen University, NL)**

Extensive chromosomal reshuffling drives evolution of virulence in an asexual pathogen.

11:00-11:30 **David Cooke (James Hutton Institute, UK)**

Tracking effector diversity in *Phytophthora infestans* populations.

11:30-11:50 **Peeters N, Carrere S., Anisimova M., Plener L., Cazalé AC & Genin S (CNRS/INRA-Toulouse, FR)**

Inventory and evolution of Type III effector proteins in the *Ralstonia*

solanacearum species complex.

11:50-12:10 **Pedersen, C., Van Themaat, E. V. L., Bindschedler, L. V., McGuffin, L., Abbott, J. C., Barton, G., Maekawa, T., Cramer, R., Lu, X., Thordal-Christensen, H., Weßling, R.2, Panstruga, R. & Spanu, P. D (Copenhagen University, DK, Max Planck Institute DE, Royal Holloway University UK, University of Reading UK, Imperial College UK, Aachen University, DE).**

The structure and evolution of barley powdery mildew effector candidates.

12:15-13:45 Lunch

13:45–15:00 Thomas Kroj and Aska Goverse

Presentation of COST Action FA1208 and discussion of upcoming events

15:30-18:30 **Posters –** Birnam House Hotel

15:30-17:00 **MC meeting –** Birnam Institute

19:00-21.30 **Reception & Buffet meal** - Birnam Institute

Friday, October 11

R genes and host targets for resistance breeding and engineering (WG4)

Chair: Vivianne Vleeshouwers

8:30-9:00 **Vivianne G.A.A. Vleeshouwers, Juan Du, Evert Jacobsen, Richard G.F. Visser (Wageningen University, NL)**

Exploiting pathogen effectors in breeding for disease resistance

9:00-9:20 **Bouwmeester, K., Wang, Y., Weide, R., & Govers F. (Wageningen University, NL)**

Lectin receptor kinases; novel leads for disease resistance in Solanaceous crops.

9:20-9:40 **Stella Cesari, Imène Abidi, Véronique Chalvon, Jean-Loup Notteghem, Jean-Benoit Morel, Ryohei Terauchi & Thomas Kroj (INRA-Montpellier, FR, Iwate Biotechnology Research Center)**

The *Magnaporthe oryzae* effectors AVR1-CO39 and AVR-Pia are recognized by the rice Nucleotide Binding-Leucine rich repeat (NB-LRR) protein RGA5 through direct interaction

9:40-10:00 **Morten Lillemo (Norwegian University of Life Sciences, NO)**

Breeding for improved resistance to *Stagonospora nodorum* blotch in wheat by elimination of sensitivity to necrotrophic effectors

10:00-10:30 Break

10:30-10:50 **Solé-Castellví, M., Reschke, M., Baufumé, S, Meynard, D., Guiderdoni, E., Koebnik, R. & Boch, J. (University of Halle DE, IRD & CIRAD Montpellier)**

Using TALENs for plant genome engineering.

10:50-11:10 **Urso, S., Biselli, C., Bagnaresi, P., Desiderio, F.,Orrù, L., Perrini, R., Crispino, L., Abbruscato, P., Piffanelli, P., Cattivelli, L. & Valè, G. (CRA-GPG Genomic Research Center IT, DRA Rice Research Centre IT, PTP Rice genomics unit IT)**

Integration of genetics and RNA-Seq approaches to dissect a durable blast resistance in rice.

11:10-11:30 **Andolfo G., Sanseverino W., Rombauts S., Van der Peer Y., Bradeen J., Carputo D., Frusciante L., Ercolano M (University of Naples Federico II IT, VIB Gent BE, University of Minnesota USA)**

Overview of tomato candidate pathogen recognition genes

11:30-11:50 **Sucher, J., Krattinger, S., Chauhan, H., Selter, S., Risk, J., Lagudah, E. & Keller, B (University of Zürich & CSIRO)**

Study of durable disease resistance Lr34 wheat gene into heterologous grass species

11:50-12:00 **Thomas Kroj & Aska Goverse**

Concluding remarks

12:00-13:30 **Lunch**

ABSTRACTS

Pathogen effectors and virulence (WG1)
Oral presentations

Identification and functional characterisation of *G. pallida* effectors and their host targets.

Mantelin, S.[1], Thorpe, P.[1,2], Cock, P.J.[1], Coke, M. [2], Urwin, P.E.[2] & Jones, J.T.[1]

[1]Cell and Molecular Sciences group, James Hutton Institute, Invergowrie, Dundee, DD2 5DA, UK.
[2]Department of Plant Sciences, University of Leeds, Leeds, LS2 9JT, UK.

The potato cyst nematode *Globodera pallida* induces complex changes in its host and effectors secreted from the pharyngeal gland cells are thought to be important in these processes. The genome sequence of *G. pallida* is now complete and a large number of candidate effectors have been identified. We have used a full life cycle transcriptome analysis, cell biology tools and tests for biological activity to prioritise effectors for further analysis. Effectors that suppress host defences have been identified. We have also screened *G. pallida* effectors against host targets of effectors from other pathogens. We have identified a NAC transcription factor as a host target of one *G. palllida* effector. NAC transcription factors are targeted by a *Phytophthora infestans* RXLR effector and were also identified as hubs targeted by *Hyalanoperonospora arabidopsidis* and *Pseudomonas syringae* by Mukhtar *et al*., (2011; Science 333, 596-601). The *G. pallida* effector is localized to the endoplasmic reticulum in plant cells and causes retention of its NAC target in the ER, preventing its relocalisation to the nucleus. This effect is not seen with other NAC transcription factors, including those targeted by other pathogens. This work further reinforces the importance of NAC transcription factors as virulence targets for diverse plant pathogens.

Two penetration specific effectors from *Phytophthora parasitica* modulate hormonal physiology of the host to facilitate infection

Mathieu Gourges

INRA/ University of Nice Sophia-Antipolis, FR, mathieu.gourgues@sophia.inra.fr

Oomycetes are crop pests which cause million dollar losses every year. There is a need for new strategies to fight against these pathogens with a limited impact on environment and human health. The molecular events occurring during the penetration of the first host cells are of pivotal importance since they guide the outcome of the interaction toward plant resistance or disease installation. A better understanding of these processes will help in identifying new methods for crop protection against oomycete pathogens. Using a transcriptome analysis, we identified a set of penetration-specific effectors (PSE) bearing a RXLR motif that are transiently accumulated during the first hours of infection. We showed that the protein encoded by two of these genes (PSE1 and PSE3) abolish plant defense responses when transiently expressed in *Nicotiana* plants. Constitutive expression of PSE1 and PSE3 proteins in Arabidopsis thaliana led to an enhanced susceptibility to *P. parasitica* infection, suggesting a role for these proteins in *P. parasitica* pathogenicity. Transgenic *A. thaliana* lines accumulating PSE1 protein showed developmental perturbations, including coiled roots, associated with altered auxin physiology. A series of experiments (hormone or inhibitor treatments, reporter lines) showed that PSE1 protein accumulation modulates auxin content of plant roots. Such modulation of the auxin content was also detected in the infected cells at the penetration site of wild type plantlets. We propose that PSE1 could favor P. parasitica virulence by locally interfering with auxin content.

Similarly, we showed that PSE3 modulates hormone signaling pathways involved in plant defense to favor infection. Our results showed that penetration specific effectors can modulate general plant functions to facilitate plant infection. Perturbation of hormone physiology was previously reported for other plant pathogens, including nematodes and bacteria, supporting the hypothesis that infection strategies from distant pathogens species could converge onto a limited set of plant targets.

Functional analysis of barley powdery mildew effector candidates and identification of their barley targets

Ali Ahmed, Carsten Pedersen and Hans Thordal-Christensen

Department of Plant and Environmental Sciences, Faculty of Science, University of Copenhagen, Denmark; aaa@life.ku.dk

The barley powdery mildew fungus (Blumeria graminis f. sp. hordei, Bgh) is a biotrophic pathogen that develops specialized feeding structures, "haustoria", inside barley cells and forms intimate relationship with the host cell. Previous studies identified around 500 Bgh effector candidates (CSEPs), which are believed to be delivered to barley cells to manipulate host defence response and facilitate susceptibility. In my PhD project, we aim to study the function of some Bgh effector candidates, identify and characterize their barley targets and investigate the localization of both effector candidates and their barley targets.
Host Induced Gene Silencing (HIGS) experiments were employed to study the function of ten effector candidates. Among them, silencing of four CSEPs displayed significant reduction in Bgh infection on barley. This suggests that these four CSEPs might have disease promoting function during barley powdery mildew biotrophy on barley. In addition, from Yeast Two-hybrid screening we identified three different small heat shock proteins Hsp17, Hsp17.9 and Hsp23.5 as barley candidate targets for these CSEPs. Small heat shock proteins have been reported to stabilize several intracellular proteins, including defense-related signalling proteins. This proposes that small heat shock proteins could be ideal targets for some Bgh effector/s to promote their survival on barley. Moreover, localization studies suggest that the mode of action of these four CSEPs could be in the plant cytosol and/or the plant nucleus.
Currently, overexpression studies of the four CSEPs are being carried out to complement the above mentioned HIGS results. Interaction of the CSEP - barley candidate targets will be confirmed using BiFC and pull-down assays. The functions of the barley targets are being studied using transient induced gene silencing and over-expression studies.

An RxLR Effector from *Phytophthora infestans* Prevents Nuclear Accumulation of two Potato NAC Transcription Factors

Hazel McLellan[1,3], Petra C. Boevink[2,3], Leighton Pritchard[2,3], Miles R. Armstrong[1,3] and Paul R.J. Birch[1,2,3].

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[2]Cell and Molecular Sciences, James Hutton Institute, Invergowrie, Dundee DD2 5DA, UK.
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The potato late blight pathogen *Phytophthora infestans* secretes a wide array of effector proteins which are thought to act in its hosts by disarming defences and promoting an environment conducive to pathogen colonisation. However, little is known about the host targets of these effectors and how they are manipulated by the pathogen. It is hoped that better understanding

of this process may lead to new opportunities for disease control. A Y2H screen identified two putative membrane-associated NAC transcription factors (TF) as the interactors of the RxLR effector Pi03192. Overexpression of Pi03192 significantly enhances *P. infestans* colonisation when expressed inside cells of the model host plant *Nicotiana benthamiana*, as does silencing of *NAC Targeted by Phytophthora* (*NTP*) *1* and *NTP2* using VIGS. The effector interacts with NTP1 and NTP2 at the endoplasmic reticulum (ER) membrane, where these proteins are localised. Transcripts of NTP1 and NTP2 rapidly accumulate following treatment with culture filtrate (CF) from *in vitro* grown *P. infestans*, which acts as a mixture of *Phytophthora* PAMPs, but significantly decrease during *P. infestans* infection suggesting that pathogen activity may prevent their up-regulation. StNTP1 and StNTP2 proteins are released from the ER membrane following treatment with *P. infestans* CF and accumulate in the nucleus, after which they are rapidly turned over by the 26S proteasome. The effector Pi03192 prevents PAMP-triggered re-localisation of StNTP1 and StNTP2 into the nucleus, revealing a novel mode-of-action for an oomycete effector.

Formation of plant tumors in the Ustilago maydis – maize interaction requires organ-specific activity of secreted effector proteins

Amey Redkar[1], Lena Schilling, Alexandra Matei, Christoph Hemetsberger, Ziba Ajami-Rashidi[1], Virginia Walbot[2] and Gunther Doehlemann[1]

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Ustilago maydis is a biotrophic smut fungus which infects all aerial organs of its host plant maize. Disease progression goes along with comprehensive reprogramming of the plant tissue which ultimately results in formation of tumors. This tumor induction is likely being triggered by small secreted proteins by the fungus, so called effectors. Given the fundamental differences between the different maize organs that are colonized by *U. maydis*, we hypothesized that the fungus deploys organ specific effectors to manipulate physiology and development of specific host tissues. To further investigate the role of individual organ specific effectors in modulating biotrophy, we performed a candidate gene approach based on transcriptional regulation and sequence divergence of effector genes. This approach identified a whole set of novel *U. maydis* effectors that contribute to fungal virulence in an organ-specific manner. One such effector is See1 (Seedling efficient effector 1), whose expression is strongly induced in seedling leaves but only weakly expressed in tassels and ears. *U. maydis* deletion mutants for *see1* show a strong reduction of tumor formation in maize seedlings but not in floral tissues. Laser scanning confocal microscopy shows that the mutant hyphae successfully enter the leaf tissue but might be blocked during pre proliferation stages in the mesophyll tissue of the leaf. Moreover, by labeling replicating DNA by 5-ethynyl-2-deoxyuridine (EdU) we observed that maize seedling colonized by Δsee1 do not show mitotic activity during infection, while cell division in leaves is specifically induced in wildtype infected host cells. In contrast, the Δsee1 mutant induces normal tumor formation in tassels and also shows the stable cell division rate in colonized anthers. To understand its organ-specific function for *U. maydis* virulence, See1 interaction partners have been identified using a yeast two hybrid screen. The presented approach provides novel insight into tissue-specific virulence strategies of a biotrophic plant pathogen.

A *Phytophthora capsici* CRN effector targets a tomato TCP transcription factor to affect its function.

Stam R.[1,2,3], Motion, G.B.[1,2,3], Boevink, P.C[2,3].& Huitema, E[1,3]

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CRNs form a large and diverse group of effector proteins in Oomycete plant pathogens. CRN proteins have been found in all plant pathogenic oomycetes sequenced to date and are thought to have expanded within the *Phytophthora* lineage. With 84 putative full length CRN coding genes identified in *P. capsici* collectively carrying 29 C-terminal effector domains, they form a complex and rich source of effector activities to study. Previously, we have shown that ectopic expression of a subset of C-terminal domains in planta, leads to their accumulation in host nuclei. These observations, together with their sequence diversity and presumed diverse activities, has led us to hypothesize that CRN effectors perturb a range of different nuclear processes.
In order to unravel the function one CRN effector, we aimed to test whether interactions identified between an *Hyaloperonospora arabidopsidis* effector HaRXLCRN15 and its *Arabidopsis* target AtTCP14 are conserved in *P. capsici* and tomato respectively. By studying the conserved CRN effector domain from *P. capsici* and its potential tomato target, we show that (i) a highly conserved CRN effector binds to the tomato transcription factor TCP14 and (ii) the presence of CRN effector activity leads to a change in TCP14 localisation. Preliminary evidence suggests that CRN induced changes in TCP14 localisation reflects its dissociation from chromatin, an event that may underpin an increase in *P. capsici* virulence in ectopic over-expression assays with both CRN and TCP14 proteins. These findings are consistent with the recent observation that besides a role in development, TCP TFs play roles in immunity and that *P. capsici* CRNs target processes associated with immunity in plants. Given that this host-target interaction appears conserved between divergent hosts and pathogens, we suggest that these interactions are important and thus form good targets to enhance immunity in plants.

Suppression of Chitin-Triggered Immunity, and Other Functions of Fungal LysM Effectors.
Kombrink, A., Sánchez-Vallet, A., Faino, L., Valkenburg, D.J., van den Berg, G. & Thomma, B.P.H.J.

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While host immune receptors detect pathogen-associated molecular patterns to activate immunity, pathogens attempt to deregulate host immunity through secreted effectors. Fungi employ LysM effectors to prevent recognition of chitin by host immune receptors, although the mechanism to compete for chitin binding remained unclear. Structural analysis of the LysM effector Ecp6 of the fungal tomato pathogen *Cladosporium fulvum* reveals a novel mechanism for chitin binding, mediated by intrachain LysM dimerization, leading to a chitin-binding groove that is deeply buried in the effector protein. This composite binding site involves two of the three LysMs of Ecp6 and mediates chitin binding with ultra-high affinity. Intriguingly, the remaining singular LysM domain of Ecp6 binds chitin with low micromolar affinity but can nevertheless still perturb chitin-triggered immunity.
Interestingly, work on *Mycosphaerella graminicola* LysM effectors revealed that these effectors may have other functions during host colonization as well. Fungal cell wall chitin is a target for plant chitinases, and *M. graminicola* LysM effectors are able to prevent hyphal lysis, whereas *C. fulvum* Ecp6 does not have this ability. Structural and biochemical analysis is performed to unravel the basis of this differential activity.

Likely, more functions for LysM effectors exist in fungal pathogens, as most of the LysM effectors produced by the vascular wilt fungus *Verticillium dahliae* appear not to play a role in pathogenesis. Only a strain-specific LysM effector that was identified in a single *V. dahliae* strain appears to be expressed in planta and is involved in virulence. Finally, the widely conserved nature of LysM effectors and their prominent role in pathogenicity predicts that plant genotypes exist that are able to (broadly) recognize LysM effectors and thus reinstall fungal immunity. Our progress on the diverse aspects LysM effector research will be reported.

Effector analysis of two different types of rice nematodes, the sedentary *Meloidogyne graminicola* and the migratory *Hirschmanniella oryzae*

G. Gheysen, L. Bauters, S. Nowak, D. Naalden & A. Haegeman*

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Our research focuses on rice as model plant to analyse the interaction with nematodes at the cellular and molecular level. To get a comprehensive overview of the compatible plant response to nematode infection, mRNA sequencing was performed on rice after nematode infection. Local infected tissue was compared with systemic tissue after infection by the root knot nematode *Meloidogyne graminicola* or the migratory nematode *Hirschmanniella oryzae* and with control tissue of the same developmental stage. One of the results is the downregulation of plant defense genes locally and systemically after root knot nematode infection. To get insight in the proteins that are secreted by nematodes into the plant in order to suppress defense and establish a successful infection, a transcriptome analysis was performed on *Meloidogyne graminicola* preparasitic juveniles and on mixed stages of *Hirschmanniella oryzae*. One of the common effectors in different types of nematodes, including the migratory species, appears to be chorismate mutase. In the future we want to extend our analyses to other types of rice nematodes (cyst nematode, stem nematode, white tip nematode).

Pathogen effectors and virulence (WG1)
Poster presentations

Identification and exploitation of effectors from barley fungal pathogen *Rhynchosporium commune*

Avrova, A.[1], Gamble, L.[1], Griffe, L.[1], Ruiz, O.[2], Münsterkötter, M.[3], Knogge, W.[4], Newton, A.[1], Birch, P.[5], Hammond-Kosack, K.E.[2] and Kanyuka, K.[2]

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The fungus *Rhynchosporium commune* is one of the most destructive pathogens of barley worldwide, especially in areas with cool temperate climate. Recent sequencing of the *R. commune* genome, and transcriptomes from germinating conidia and an early time point during barley colonization, provided a unique opportunity to identify the putative effector population

mediating interactions with the host plant. Genome comparison of 9 *R. commune* strains allowed rapid prediction of candidate effectors less variable in *R. commune* populations. Less variable effectors are more likely to be essential for pathogenicity. Candidate effectors have been transcription profiled during barley infection using qRT-PCR. Targeted gene disruption of candidate effectors, highly abundant early during the infection, will help to determine their importance for pathogenicity. We are also using a Barley stripe mosaic virus (BSMV) mediated transient over-expression system to aid functional characterization of predicted effectors. This system allows exploitation of effectors for screening of barley accessions for R-gene mediated recognition of individual effectors in an attempt to identify novel sources of potentially durable resistance to *R. commune*.

Hunting avirulence effectors in the wheat powdery mildew *Blumeria graminis f. sp. tritici*.
Bourras, S., Mc Nally, K., Roffler, S., Wicker, T., Parlange, F., Ben David, R., Oberhänsli, S, Praz,C., Menardo, F., Shatalina, M., Buchmann, J, Wicker, T. & Keller, B.

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The powdery mildew *Blumeria graminis f. sp. tritici* (Bgt) is a major fungal pathogen of wheat. Resistance of wheat to Bgt is believed to follow a gene-for-gene model involving Pm resistance genes from the plant and AvrPm avirulence effectors from the pathogen. Despite the identification of a promising candidate avirulence effector (AvrPm3f1), the Pm-AvrPm interaction remained elusive. During the past two years, tremendous efforts were undertaken to indetify avirulence effectors. Numerous resources were produced by us, including the Bgt reference genome, the genome sequences of 19 natural isolates, two mapping populations of 150+ progenies each and RNA sequencing data from Bgt infected wheat. We employed highthroughtput genotyping (KASPar technology), bulk segregant sequencing (Illumina technology) along with comparative genomics, effectors genomics and genome-wide SNP association studies. Our data indicate that presence/absence polymorphism of a single effector gene is not sufficient to explain the virulence/avirulence polymorphism on Pm differential wheat lines. In the case of the Pm3-AvrPm3 pair, we found that the interaction functions in an allelic manner and often one Pm3 allele recognizes two AvrPm loci, suggesting a tripartite model involving one resistance protein and two avirulence effectors. The AvrPm3f1 locus is common to all interactions involving the Pm3 alleles investigated so far. We were also able to identify a second candidate avirulence effector, AvrPm3f2_7, as being the potential second effector, besides AvrPm3f1, interacting with the f allele of Pm3. As previously observed in the AvrPm3f1 locus, the AvrPm3f2_7 locus also contains a cluster of effectors belonging to the same family as the candidate avirulence effector. Additionally, we are analyzing the AvrPm2 locus to identify the avirulence effector partner of Pm2 resistance gene. Currently, 11 Pm-AvrPm interactions are at different stages of investigation in our group.

Cytokinins: novel effectors of *Magnaporthe oryzae*?
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The role of cytokinins in plant resistance has been demonstrated in a few plant-pathogen interactions (Matsumoto, 1980; Siemens et al, 2006; Choi et al, 2010; Argueso et al, 2012). The role of these hormones is still unclear as in some systems it promotes susceptibility (Siemens et al, 2006) whereas in others it promotes resistance (Choi et al, 2010). Recently, Jiang et al (2012)

showed that the rice blast fungus, *Magnaporthe oryzae*, produces and secretes some cytokinins but the role of these fungal-derived hormones is not established. This production of cytokinins by the blast fungus could confer an advantage to the pathogen during the earlier stages of infection since cytokinins could trigger an uptake of nutrient in infected cells. We have tested an exogenous application of cytokinins before infection. Rice plants pre-treated with cytokinins display a decrease in the number of lesions due to the blast fungus. This effect is dependent of the timing of treatment but not of the isolates tested. Gene expression analysis indicates that rice plants pre-treated with cytokinins display enhanced defense response in the presence of the fungus. This increased resistance of rice leads to a delay of the pathogen growth, already visible at 48h after inoculation. A strong increase of HR is accompanying this enhanced resistance. In addition, experiments with rice mutant plants putatively over-accumulating cytokinins suggest that endogenous cytokinins are required for resistance. In plants and bacteria the first step of biosynthesis of cytokinins is catalysed by an Isopentenyl Transferase (Sakakibara, 2006) but in fungi this pathway is poorly understood. We identified a single gene coding for this enzyme in *M. oryzae* genome and are producing loss-of-function mutants for this gene. Analysing the pathogenicity of these *M. oryzae* mutants should allow clarifying the ambiguous role of cytokinins during rice infection.

Characterisation of novel secreted proteins and their potential role in cyst nematode parasitism

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Plant parasitic nematodes comprise several groups, the most economically damaging of these are the sedentary endoparasites, causing losses to agriculture worth in excess of £75 billion per year. Sedentary endoparasitic nematodes are obligate biotrophs and modify host root tissue, using a suite of effector proteins, to create a feeding site that is their sole source of nutrition. Using transcriptome sequences across the life cycle of the cyst nematode *Globodera pallida,* mapped to its recently assembled genome sequence, we have identified a range of novel putative secreted proteins potentially involved in the feeding process. We have characterised a large gene family that is highly diverse between individuals of the same population, and implicate its role in cyst nematode feeding. Expression in the amphid sheath cells has been confirmed by *in situ* hybridisation. *In planta* double stranded RNA expression targeting this gene family results in approximately 80 % reduction in total nematode infection. Representatives of this gene family have been identified in other cyst nematode species.

Search for virulence-associated factors in *Verticillium albo-atum*

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WGS of three mild and three highly aggressive (lethal) strains of *Verticillum albo-atrum*, a soil born vascular pathogen, was employed to study the mechanisms behind the development of the increased virulence of the lethal strains and to detect virulence-associated factors. *De-novo* assembly of the reference genome (35.6 Mb) and reference mapping of the other five genomes was performed, revealing a 0.5 Mb genomic region common to all three lethal strains and absent

in the mild strains. Gene prediction tools supported by Exonerate protein alignments and RNA-seq analysis resulted in 9858 gene models. In silico identification and characterization of effectors is currently underway. Around 90 gene models were predicted in the lethal specific region, with additional evidence of a few regions being expressed but not predicted by the software tools. Targeted deletion analysis has been introduced to set up an efficient system for functional characterization of candidate genes. Ten transcripts of lethal strains with the highest expression level in xylem simulating medium and five *in planta* expressed fungal transcripts as detected by proteomic and transcriptomic analysis are currently being tested using a *V. albo-atrum* and *Nicotiana benthamiana* pathosystem.

Identification of effectors in wheat and stripe rust (Pst) inter¬action

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The loss of efficiency of resistance (R)-genes due to appearance of new virulent strains is a common problem in many diseases. However, the emergence and rapid spread of virulent strains of the stripe rust fungus makes this disease particularly threatening for global wheat production. Therefore, the fight against stripe rust epidemics requires an understanding of how pathogen characteristics interact with characteristics of the host plant (Hovmøller et al., 2011). We will isolate hautoria of both aggressive and non-aggressive isolates for subsequent RNA-seq using ConA affinity chromatography method (Hahn et al., 1992). The results of RNA-seq will provide information of the effector repertoire of Pst and allow us to predict putative cytoplasmic effectors. These effector candidates will be knocked down one-by-one in wheat leaves by agro infiltration (Penwar et al. 2013) to test if they indeed have effector function. However, agro infiltration in cereals has hitherto been regarded as very difficult or impossible and if it turns out to be impossible to repeat the newly published method, we will implement a VIGS-procedure instead (Yuan et al. 2011). Well-described rust proteins such as the hexose transporter, HXT1 (Voegele et al. 2001), and amino acid transporter, AAT2 (Hahn et al. 1997), as well as the rust transferred protein 1, RTP1 (Pretsch et al. 2012), known to be highly expressed in haustoria and likely important for biotrophy will be cloned and tested in the transient assay.

Identifying and characterizing root-knot nematode candidate effectors

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Plant-parasitic nematodes are a huge agricultural problem on many of the world's main food crops, and some of the most damaging of the plant-parasitic nematodes are the root-knot nematodes (*Meloidogyne* spp). To establish infections across many plant species, the nematode presumably secretes a large repertoire of effectors that suppresses plant defences and/or alters host cell physiology to form the successful feeding cells. Using the known root-knot nematode secretome and genome(s), we identified several novel proteins from the nematode and are currently undertaking a multistep approach that will help us to prioritize these candidate proteins for further mechanistic investigations. In one approach, we are utilizing a novel effector screen in which nematode genes are heterologously expressed in the bacterial pathogen *Pseudomonas syringae*. In addition, we are following the gene expression profiles in various nematode life-stages to further narrow our candidate list. Following this pipeline for new

effector discovery, we are now in the early steps in characterizing a select list of root-knot nematode proteins and their possible roles in the compatible interaction with *Arabidopsis thaliana*. By using the genetic and genomic resources available for *Arabidopsis*, we hope to further our understanding of the nematode's relationship with the plant.

Characterization of root-knot nematode effectors targeted to the nuclei of giant cells
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Root-knot nematodes are obligate endoparasites that maintain a biotrophic relationship with their hosts over a period of several weeks and induce the differentiation of root cells into specialized multinucleate feeding cells, named giant cells. Nematode effectors synthesized in the oesophageal glands and injected into the plant tissue through the syringe-like stylet play a central role in giant cell ontogenesis. In a search for nematode effectors targeted to the giant cell nuclei, we used bioinformatics and comparative genomics on expressed sequence tag datasets to identify *Meloidogyne incognita* genes encoding proteins potentially secreted upon the early steps of infection. We identified genes specifically expressed in the oesophageal glands of parasitic juveniles that encode predicted nuclear and secreted proteins. One of these genes, Mi-EFF1 is a pioneer gene that has a predicted nuclear localization signal but no similarity in databases. We demonstrated that *M. incognita* injects Mi-EFF1 within the feeding cells. Mi-EFF1 is targeted into the nuclei of the feeding cells where it may manipulate nuclear functions of the host cell.

Plant proteins and processes targeted by effectors (WG2)

Oral Presentations

Pumped up on (brassino)steroids: the late blight pathogen manipulates the BR pathway in potato.
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Phytophthora infestans is the oomycete responsible for late blight; the most challenging disease of potato crops globally. The pathogen secretes effector proteins into the plant, capable of modifying host cell processes to help establish disease. Some plants have evolved resistance genes to recognise specific effectors, allowing detection of pathogen invasion and initiation of plant defences.
P.infestans effector PiAVR2 is recognised in potatoes by a family of R2-like NB-LRRs. We have previously shown that PiAVR2 interacts with potato phosphatase BSL1, which regulates a key step in brassinosteroid (BR) pathway signal transduction and that both are required for R2 activation. The BR pathway has well-characterised links to growth, development and abiotic stress, and more recently to disease resistance particularly in the model plant Arabidopsis. Our on-going research is to characterise both the intricate interaction between effector, target and resistance protein, as well as the broader impact of PiAVR2 on the host plant. In addition, we

have identified two other unrelated *P.infestans* RXLR effectors that each interact with another host protein that is a candidate for functioning at additional steps in the BR pathway. Understanding the biological 'motives' of these effector proteins could prove informative to crop breeding efforts, not only in the case of late blight but in other plant-pathogen interactions implicating the BR pathway.

***Phytophthora infestans* RXLR effector AVR3a targets a GTPase involved in plant immunity**

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Phytophthora infestans, the causal agent of potato late blight, secretes effector proteins to overcome plant immunity. An essential RXLR effector, AVR3a, suppresses the cell death induced by *P. infestans* INF1 elicitin, a conserved secreted protein with features of pathogen-associated molecular patterns (PAMPs). SERK3/BAK1 a co-regulator of several surface receptors is a key element for *P. infestans* pathogenicity and for INF1 cell death. We investigated the extent and the specificity to which AVR3a affects PTI signaling that requires the modulator SERK3/BAK1 in *N. benthamiana*. We found that all AVR3a variants suppressed PAMP-induced reactive oxygen species production (ROS) and induction of gene expression. Using co-immunoprecipitation and mass spectrometry analysis, we identified a plant GTPase involved in cellular trafficking that associates with all variants of AVR3a. Transient over-expression of this GTPase enhances *P. infestans* growth and accumulates around haustoria during infection. In addition, altering the GTPase cellular concentration impairs ROS production. These results suggest that AVR3a associates with a GTPase to manipulate host defenses and supports *P. infestans* virulence. Further work is underway to dissect the underlying mechanisms.

A novel host target of Phytophthora infestans RXLR effector

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The oomycete plant pathogen *Phytophthora infestans*, the causal agent of potato and tomato late blight, secretes a significant amount of RXLR-effectors to establish infection. RXLR-effectors are able to manipulate host defence by suppressing PAMP-triggered immunity (PTI) and effector-triggered immunity (ETI). Several RXLR effectors act as Avirulence (Avr) proteins since they are recognized by cognate host resistance (R) proteins and induce plant ETI. How RXLR-effectors supress or elicit host defence is largely unknown. We have identified Avr1, an RXLR-effector that can suppress plant PTI and that triggers a hypersensitive response (HR) upon recognition by the potato resistance protein R1. In order to understand how Avr1 functions, a yeast-2-hybrid assay was conducted to search for host targets of Avr1. In this way we identified the host exocyst component Sec5 as an interactor of Avr1. The plant exocyst is involved in vesicle trafficking and known to play a role in the immune response against pathogen attack. Silencing of Sec5 resulted into a compromised R1-mediated resistance. Further functional analysis showed that Sec5 is crucial for proper plant defence and plant exocytosis.

Interactions of HopQ1, a type III secretion effector from Pseudomonas syringae, with host 14-3-3 proteins

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HopQ1 (for Hrp outer protein Q), a TTSS effector secreted by *Pseudomonas syringae pv. phaseolicola*, promotes the development of halo blight in bean. However, when this same effector is injected into tobacco cells, it is recognized by the immune system and prevents infection. Although the ability to synthesize HopQ1 determines host specificity, the role it plays inside plant cells remains unexplored. We have previously shown that after expression in planta, HopQ1 co-purified with host 14-3-3 proteins. The physical interaction between HopQ1 and 14-3-3a was confirmed using FRET-FLIM techniques. Mass spectrometric analyses detected specific phosphorylation of the canonical 14-3-3 binding site present in HopQ1. Substitution within this motif abrogated the interaction and led to altered subcellular localization of HopQ1. Moreover, the mutated HopQ1 protein showed reduced stability in planta. We are currently analyzing structures of monomeric and oligomeric forms of HopQ1 and the complex of HopQ1 with 14-3-3a, using MALS (multiangle light scattering analysis) combined with size exclusion chromatography and SAXS (small angle X-ray scattering) techniques supported by molecular modeling. Results so far will be presented.

Analysis of barley cell vesicle trafficking after powdery mildew invasion reveals the identity of the extrahaustorial membrane

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Certain filamentous microbes of major economic importance for agriculture are extreme in their intimacy with plants. Structures of then literally exist inside living plant cells, where they benefit from a continuous supply of host nutrients. This intimacy is made possible by a plant cell-generated, but microbe effector-guided, membrane that separates the structures from the host cytosol. Despite their obvious importance, the nature and source of these membranes remain enigmatic.
Many of these microbes are pathogenic, in which cases the structures introduced into the host cells are called haustoria. We have undertaken a study of barley cell vesicle trafficking components and membrane markers in a search for the identity of the extrahaustorial membrane (EHM) generated around powdery mildew haustoria. Long-time exposure to the membrane dye, FM4-64, a marker for endocytic activity that gradually becomes integrated in endosomal and vacuolar membranes, never led to staining of the EHM. This indicated that the EHM is derived from membranes of the early secretory pathway. Use of dyes and protein markers for specific membranes confirmed this observation. Subsequent interference with selected vesicle trafficking pathways after over-expression of mutated proteins has allowed us to determine from which membrane compartment the EHM is likely derived.

Secreted venom allergen-like proteins of plant-parasitic nematodes modulate defence responses in host plants

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The venom allergen-like proteins form a family of effectors that seems to be conserved among all parasitic nematodes of plants and animals studied to date. Recently, we have shown that the venom allergen-like protein of the potato cyst nematode *Globodera rostochiensis* Gr-VAP1 interacts with the apoplastic cysteine papain-like proteases Rcr3pim of *Solanum pimpinellifolium*. Gr-VAP1 and Rcr3pim are both required to activate defence-related programmed cell death and resistance to nematodes mediated by the extracellular plant immune receptor Cf-2 in tomato. Thus, Gr-VAP1 is able to trigger defence responses in a host plant of *G. rostochiensis*, but the virulence function of Gr-VAP1 or of any other venom allergen-like protein of an animal- and plant-parasitic nematode is not known. A specific knock-down of Gr-VAP1 expression in *G. rostochiensis* showed that the effector is indeed important for virulence of infective juveniles in host plants. Similarly, the ectopic expression of venom allergen-like proteins in transgenic plants alters their response to nematodes and other plant pathogens. RNAseq analysis of these transgenic plants has shed light on the molecular mechanisms underlying the virulence function of venom allergen-like protein of plant-parasitic nematodes in plants.

Rice SWEETs as virulence targets for Xanthomonas TALENs.
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Phytopathogenic bacteria of the genus *Xanthomonas* inject type III effectors directly into the cytoplasm of the plant cell. The TAL (transcription activator like) effector family are important virulence factors that can act as transcription factors and activate plant gene expression to the benefit of the pathogen. TAL effectors contain a type III secretion and translocation signal, nuclear localisation signals (NLSs), an acidic activation domain and a central region of tandemly arranged, nearly identical repeats. Although it is known that the composition of the repeat region mediates the DNA binding in a specific and predictable manner little is known about biological virulence targets. So far, three genes of the rice SWEET gene family have been identified as virulence targets of different TAL effectors from *Xanthomonas oryzae pv. oryzae* (Xoo). To test which SWEET members can potentially function as virulence targets we constructed artificial TAL effectors (ArtTALs) to individually target 20 SWEET orthologs in rice. The ArtTALs were used as designer virulence factors in an in vivo assay to test whether they support Xoo virulence. Our results demonstrate that five phylogenetically close SWEET proteins support Xoo virulence. These SWEET proteins potentially mediate sucrose efflux from the phloem parenchyma cells to the apoplast. Therefore, the induction of these genes might have a positive impact on nutrient availability for the pathogen in the apoplast.

***Xanthomonas campestris* type III effector XopQ interacts with tomato and pepper 14-3-3 isoforms to suppress effector-triggered immunity**
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Effector-triggered immunity (ETI) to host-adapted pathogens is associated with the elicitation of a rapid cell death at the site of infection. The plant-pathogenic bacterium *Xanthomonas campestris pv. vesicatoria* (Xcv) interferes with plant cellular processes by injecting effector proteins into host cells through the type III secretion system. Here, report that the *X. campestris* effector XopQ suppresses cell death induced by components of the MAP kinase cascade MAP3Kα/MEK2/SIPK and by several R/avr gene pairs. Inactivation of xopQ by insertional mutagenesis revealed that this effector inhibits the ETI-associated cell death induced by avirulent Xcv strains in resistant pepper, and promotes bacterial growth in resistant cultivars of pepper and tomato. Protein-protein interaction studies indicated that XopQ interacts with the tomato 14-3-3 isoform TFT4 and homologous proteins of other plant species. Remarkably, a mutation in the 14-3-3 phosphoserine binding site of XopQ was required for the interaction of the effector with TFT4 and for its virulence function. Consistent with a role in plant ETI, TFT4 gene expression was induced during the incompatible interaction of tomato and pepper plants with Xcv. In addition, silencing of the TFT4 homolog CaTFT4 in pepper enhanced susceptibility to avirulent Xcv strains and delayed the appearance of ETI-associated cell death. Together, our results indicate that the XopQ virulence function is to suppress ETI and immunity-associated cell death by interfering with the function of plant TFT4 proteins, which represent important components of ETI and bona fide targets of XopQ.

An endocytotic pathway targeting the vacuole is re-routed towards pathogen penetration sites in Plants.

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To enable parasitism and symbiosis, plant-associated organisms intimately interact with plant cells often through specialized cellular structures. For example some fungal and oomycete pathogens form accommodation structures termed haustoria that invaginate the host cell plasma membrane to deliver pathogenicity effector proteins and acquire nutrients. In response, the attacked plant cell synthesizes a new membrane surrounding the haustoria called the extrahaustorial membrane (EHM), which differs from plasma membrane in various aspects. Moreover, the plant cell undergoes significant cellular reorganization involving organelle relocation and polarized secretion of anti-microbial molecules at contact sites.
As a countermeasure effective pathogens such as the Irish potato famine pathogen *Phytophthora infestans* secrete effector proteins to neutralize such innate resistance responses. However, molecular mechanisms underlying how pathogens perturb host endomembrane traffic, intracellular pathways required for the EHM formation, and how macromolecule transport at this interface is regulated are poorly known.
The role of focal secretion in immunity has been difficult to dissect using standard genetic approaches because mutants often show pleiotropic effects that perturb plant development. As an alternative approach we focused on unraveling the mechanisms underlying EHM biogenesis by using bio imaging techniques and effectors as molecular probes that mark the EHM. These experiments revealed that: (i) a selective vacuolar route to the EHM for protein transport; (ii) some surface immune receptors might traffic to the EHM via late endosomes. In summary our work indicates that effectors can be used as molecular probes to unravel unknown facets of focal immunity and enable us to visualize differences between the haustoriated cells and neighboring uninfected cells.

Plant proteins and processes targeted by effectors (WG2)

Poster Presentations

Searching for the mechanism of endomembrane sequestration in plant-pathogen interaction

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The causal agent of barley powdery mildew, *Blumeria graminis f.sp. hordei* (Bgh), is an intracellular biotroph that depends on formation of a host-derived endomembrane around its feeding structure, the haustorium. Because this membrane is vital for the fungus, it is likely that Bgh highjacks the host endomembrane traffic in order to establish the extrahaustorial membrane (EHM). Effector-mediated endomembrane sequestration is still elusive for plant interacting microbes and the particular membrane nature of the EHM is unknown. Our work focuses on understanding how the barley powdery mildew fungus induces formation of the EHM. A Bgh cDNA library was generated at a critical time point for EHM formation. This library was ectopically expressed in yeast and screened for a vacuolar protein sorting (vps)-dysfunctional phenotype. Four candidate effector proteins were identified and their relevance for fungal virulence was confirmed for two of them using RNAi. Interactors of these two effector candidates are currently being screened in a nuclear yeast two-hybrid. Identification of effectors and their targets required for host endomembrane sequestration will not only help elucidating the origin of the EHM, but also give insights on how these pathogens are able to establish and maintain the intracellular interface within their hosts.

Genome-Wide Innate Immune Responses to *Fusarium oxysporum* and Tomato mosaic virus in Tomato

Andolfo G., Ferriello F., Tardella L., Sigillo L., Frusciante L., Ercolano M.R.

In recent years, owing to the availability of high throughput biological data and computational resources, interest in modeling of plant-pathogen interactions has grown. Gene expression approaches constitute a starting point for investigating a plant–pathogen systems. In order to identify a set of genes of interest in tomato plants infected with *F. oxysporum f. sp. lycopersic*i (Fol) and Tomato Mosaic Virus (ToMV) a transcriptional analysis was performed. Tomato genes differentially expressed upon inoculation with Fol and ToMV were identified at 2 days post-inoculation, using an un-inoculated sample as reference. A large overlap in differentially expressed genes throughout the two incompatible interactions was found. However, GO enrichment analysis evidenced specific categories enrichment in both interactions. Response to ToMV seems more multifaceted, since more than 70 specific categories were enriched versus the 30 detected in Fol interaction. In particular, virus infection stimulated the production of an invertase enzyme that is able to redirect the flux of carbohydrates acquisition whereas Fol induced a homeostatic response to prevent the attempt of fungus to kill the cells. Genome arrangement of expressed genes along chromosome was shown to be important for explaining transcriptional changes.

Multiple layers of barley powdery mildew triggered nonhost resistance in wheat genotypes

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The majority of plants are immune against majority of plant pathogenic microbes as they express nonhost resistance to pathogenic attacks. Since this type of resistance is considered to be not only more durable than race-specific resistance, but also effective against a large scale of pathogens our aim was to investigate the mechanisms of barley powdery mildew triggered immunity in various wheat genotypes.
As nonhost plant, seven winter wheat cultivars/lines, as host plants near isogenic lines of spring barley cultivars Ingrid and Pallas with or without Mla, Mlg or mlo resistance genes were used. *Blumeria graminis f. sp. hordei* (Bgh) race A6 served as pathogen.
To our surprise microscopic investigations already in germination of powdery mildew conidia showed striking differences on various wheat genotypes. While on host barley cv. Ingrid and on one of the wheat genotypes conidia germinated almost by 100%, on another wheat genotype the germination was only about 50%! In addition, in the first five days there were practically no papillae formation and no hypersensitive response (HR) on the host barley cultivar, but on nonhost wheat genotypes papilla formation ranged from 37 to 74%, and HR induction from 8 to 31% of the total tested conidia. Interestingly enough, in some cases even elongated secondary hyphae and mesophyll cell HR were found in nonhost reactions indicating the multiple layers of PAMP and effector triggered immunity. Induction of HR was accompanied by a stronger, papillae formation by a weaker accumulation of the reactive oxygen species H2O2, as demonstrated by DAB staining. It is noteworthy that a certain degree of resistance was induced by first inoculation with Bgh A6 practically in all of the wheat genotypes to a second inoculation with virulent races of wheat leaf rust, as it is expressed in a reduction of the number of rust colonies.
Considering that it was reported recently, that a superfamily of barley powdery mildew effector candidates show structural affinities to ribonucleases (RNases), and in our previous experiments RNases were activated more strongly in compatible than in incompatible wheat-rust interactions, changes in RNase activities were also investigated by PAGE and spectrophotometric assays. Indeed, in wheat the virulent powdery mildew induced the larger, the barley powdery mildew the smaller increase in activities of RNase isoenzymes with about 100, 75, 16 and 12 kD. The possible role of RNases as virulence factors is further investigated.

Genome-wide transcriptome analysis of the *Arabidopsis thaliana/Phytophthora parasitica* interaction: towards the characterization of genes involved in plant susceptibility to soilborne oomycetes.

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Oomycetes from the genus *Phytophthora* are fungus-like pathogens that are devastating for agriculture and natural ecosystems. Due to their particular physiological characteristics, no sustainable management strategy of these microorganisms is currently available. Deciphering molecular mechanisms that govern interactions between *Phytophthora* spp. and their host plants may thus provide clues to develop new, efficient plant protection approaches. In this context, our laboratory aims at understanding the molecular events underlying the onset of the compatible interaction between plant roots and *Phytophthora* species. For this purpose, we took advantage of a system involving the model plant Arabidopsis thaliana and the wide host range pathogen, *Phytophthora parasitica*. We monitored *A. thaliana* transcriptome modulations from root penetration by *P. parasitica* motile zoospores to the switch from biotrophy to necrotrophy. We showed that the interaction transcriptome is highly dynamic, and that many biological processes are regulated as soon as the first contact between plant roots and the oomycete

occurs. Among them, responses involving the hormones ethylene (biosynthesis and response) and auxin (cellular transport) are highly modulated. To identify plant functions that are manipulated by *P. parasitica*, we selected *A. thaliana* candidate genes that show a significant transcriptional up-regulation during the first hours of infection. The characterization of corresponding knock-out mutants revealed two genes contributing to susceptibility, and three to resistance of *A. thaliana*. These results will be presented and their potential use for plant protection purposes will be discussed.

Expression of *Pseudomonas syringae* type III effectors in yeast under stress conditions reveals that HopX1 attenuates activation of the high osmolarity glycerol MAP kinase pathway

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The Gram-negative bacterium *Pseudomonas syringae pv. tomato* (Pst) is the causal agent of speck disease in tomato. Pst pathogenicity depends on a type III secretion system that delivers effector proteins into host cells, where they promote disease by manipulating processes to the advantage of the pathogen. Previous studies identified seven Pst effectors that inhibit growth when expressed in yeast under normal growth conditions, suggesting that they interfere with cellular processes conserved in yeast and plants. We hypothesized that effectors also target conserved cellular processes that are required for yeast growth only under stress conditions. We therefore examined phenotypes induced by expression of Pst effectors in yeast grown in the presence of various stressors. Out of 29 effectors tested, five (HopX1, HopG1, HopT1-1, HopH1 and AvrPtoB) displayed growth inhibition phenotypes only in combination with stress conditions. Viability assays revealed that the HopX1 effector caused loss of cell viability under prolonged osmotic stress. Using transcription reporters, we found that HopX1 attenuated the activation of the high osmolarity glycerol (HOG) mitogen-activated protein kinase (MAPK) pathway, which is responsible for yeast survival under osmotic stress, while other MAPK pathways were mildly affected by HopX1. Interestingly, HopX1-mediated phenotypes in yeast were dependent on the putative transglutaminase catalytic triad of the effector. This study enlarges the pool of phenotypes available for the functional analysis of Pst type III effectors in yeast, and exemplifies how analysis of phenotypes detected in yeast under stress conditions can lead to the identification of eukaryotic cellular processes affected by bacterial effectors.

Novel insights into the molecular mechanisms underlying aphid host range

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Aphids are devastating plant sap-feeding insects. These insects cause direct feeding damage and transmit the majority of plant viruses, resulting in significant yield losses, particularly in staple food crops. Interestingly, most aphid species are restricted to one or few host plants. However, some species, many of which are of agricultural importance, can infest a wide range of plant species. An important observation is that aphids spend a considerable time on non-host plants, where they probe the leaf tissue and secrete saliva, but for unknown reasons are unable to ingest phloem sap. These findings suggest that aphids, like plant pathogens, interact with non-

host plants at the molecular level, but potentially are not successful in suppressing plant defenses and/or releasing nutrients. Recent work suggests that aphids, like plant pathogens, secrete effectors into their host plants to manipulate host cell processes and impact the ability to infest plants. Our project aims to investigate the role of plant cellular processes, as well as aphid proteins (including effectors) in determining aphid host range.
We are comparing interactions of economically important aphid species, *Myzus persicae* (green peach aphid) *Myzus cerasi* (black cherry aphid) and *Rhopalosiphum padi* (bird cherry oat aphid) with host and non-host plants. More specifically, we are characterizing host versus non-host plant responses upon aphid interaction by investigating the activation of plant defences, and changes in plant gene expression and cellular organization. In addition, we are characterizing the role of aphid candidate effectors from the broad host range aphid *M. persicae* in manipulation of host responses as well as affecting host range. Understanding the role of both plant and aphid proteins in different types of interactions will provide us with key insight that are needed to develop novel strategies to control aphid infestations.

The *Phytophthora infestans* RXLR effector PexRD2 interacts with host MAP3Kε to suppress plant immunity related signalling.

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The genome of causative agent of late blight in potato and tomato, *Phytophthora infestans*, encodes > 500 RXLR-type effector proteins. A subset of these effector proteins are expressed, secreted and delivered to host cells during infection and are thought to interfere with host cell processes to facilitate pathogenesis. There is significant interest the identity of the host cell targets of RXLR effectors and the molecular mechanisms by which effectors perturb their activity. Understanding how effectors function may suggest novel approaches for engineering disease resistance. Recently, the first protein crystal structures of RXLR effectors were determined, identifying a common fold likely to be adopted by many of these proteins despite a lack of significant sequence identity. One RXLR effector for which a structure was determined was PexRD2. With knowledge of the structure, we decided to define the host cell targets for this protein to better understand its function. A Y2H screen revealed an interaction with MAP3Kε, a protein kinase previously identified as having a role in plant immunity. We find that both overexpression of PexRD2 and knockdown of MAP3Kε by VIGS in the model plant *N. benthamiana* enhances growth of the pathogen. We have also shown that PexRD2 is able to suppress cell deaths either triggered by or dependent on MAP3Kε in *N. benthamiana*. Structure-based mutations in PexRD2, which no longer interact with MAP3Kε, no longer promote pathogen growth or suppress the cell deaths assayed. We suggest that MAP3Kε is a component of a MAPK cascade of relevance to plant immunity that is targeted by PexRD2. MAPK cascades are well established as targets of bacterial effectors. Our study is the first demonstration of an oomycete effector that directly interacts with a host kinase to interfere with its signaling activity.

The dynamics of protein complexes interacting with regulatory regions of genes regulated on nematode infection

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The compatible host reaction to plant parasitic nematodes is strictly regulated via diverse signalling pathways and results in the development of specialized feeding structures in parallel with the suppression of defence response. The resulting dynamics of gene transcription was documented by several approaches. In order to monitor factors directly engaged in this gene regulation we develop a proteomic approach aiming to apply LC-MS/MS protein identification directly to DNA bound proteins. Tomato roots were infected with *Globodera rostochiensis* larvae. Proteins were isolated from dissected syncytia and subsequently enriched in nuclear fraction. Proteins were bound to fragments of regulatory regions of nematode induced genes and analyzed on LC-MS/MS. Resulted spectra were preprocessed with Mascot Distiller and proteins were identified by tomato and nematode specific databases search using Mascot search engine. Applied methods revealed qualitative differences between DNA-bound proteins of infected and control tomato roots. Especially a few new putative transcription factors were found in infected root protein sample. Our search did not show any direct interactions of analyzed promoters with nematode proteins based on currently available databases. The applied method is promising, however further optimization is required.

Contribution of pathogen and pest effectors to virulence and host range

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Plant parasites require close associations with their host for their survival. Oomycete plant pathogens form intracellular feeding structures called haustoria, while nematodes and aphids use stylets to interact with their hosts. Despite variation in associations, a common theme is that plant parasites secrete effectors to suppress defences and/or promote nutrient release. Evidence suggests that effectors from distinct pathogens target conserved components of the plant immune network. Our project investigates how aphids target host cells and how their effector-mediated host targeting strategies compare to those of nematodes and oomycetes. In addition, we aim to understand the molecular mechanisms underlying host range by using the aphid-*Arabidopsis* model system. For the latter, we are screening 527 *Arabidopsis* inbred lines for differences in aphid survival using a host-adapted form of the aphid species *Myzus cerasi* (black cherry aphid) and nonhost aphid species *Rhopalosiphum padi* (bird cherry oat aphid). To investigate overlap in host targeting strategies, we will screen aphid effectors from the broad host range aphid *Myzus persicae* against a *Nicotamania benthamiana* Yeast-2-Hybrid (Y2H) library for host target identification, and perform functional characterization studies in planta. Five aphid effectors have now been localised in planta as GFP fusion proteins. Interestingly, one of our candidate effectors shows localization into the host nucleus. We are currently testing whether any of these aphid candidate effectors alter aphid performance upon transient over-expression in planta.

Also, a Y2H library is currently being generated using RNA from dissected *M. persicae* heads, which contain the salivary glands. This library will be screened against known plant pathogen effector targets to identify putative interacting aphid effectors and gain insight into common strategies of distinct plant parasites.

The transcription factor BZR1 regulates the trade-off between plant growth and immunity

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Because of resource limitations, a trade-off between growth and immunity exists in plants, leading to the idea that these processes are controlled by interconnected signaling pathways. This trade-off needs to be finely regulated to ensure proper allocation of resources in an efficient and timely manner upon effective integration of environmental cues. The first layer of plant immunity is based on the perception of pathogen-associated molecular patterns (PAMPs) by surface-localized pattern-recognition receptors (PRRs), leading to PAMP-triggered immunity (PTI). Interestingly, several PRRs (such as FLS2 and EFR) are leucine-rich receptor kinases and rely on the regulatory leucine-rich receptor-like kinase BAK1 for signaling. Since BAK1 is a positive regulator of brassinosteroid (BR)-mediated growth, it was often postulated that an antagonism may exist between the BR- and PAMP-triggered signaling pathways. While such antagonism could be recently demonstrated, the exact underlying molecular mechanisms remain controversial. We have confirmed that the BR-PTI antagonism does not occur at the level of the BAK1-containing receptor complexes. Instead, we reveal that the BR-responsive transcription factor BZR1, but not BES1, is required and sufficient for suppression of PTI. Activation of BZR1 impacts several PTI outputs, including resistance to non-adapted bacteria. Meta-analysis indicates that BZR1 induces the expression of several WRKY transcription factors that negatively control early PTI signaling. Notably, BZR1 associates at the protein level with one of the members of the WRKY family to mediate the antagonism between BR and PTI signaling. We further show that the BZR1-mediated suppression of PTI is particularly relevant under conditions in which fast growth is required, such as etiolation. We propose a model in which BZR1 acts integrating environmental cues and generating appropriate outputs to regulate the trade-off between growth and immunity. This role of BR signaling is in line with recent works unveiling this pathway as a target of pathogens.

A geminiviral effector interferes with jasmonate signalling and plant defence responses

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Viruses must create a suitable cell environment and elude defence mechanisms, which likely involves interactions with host proteins and subsequent interference with or usurpation of cellular processes. Geminiviruses are insect-transmitted small DNA viruses infecting a wide

range of vegetable and field crops worldwide. Due to limited coding capacity, geminiviruses must rely on the plant machinery for the accomplishment of their infection. C2 is a multifunctional geminiviral protein important for pathogenicity, which has been shown to interfere with the function of the ubiquitin E3 ligase SCF complexes in the cell, affecting multiple plant responses. Here, we describe that C2 from the begomovirus Tomato yellow leaf curl Sardinia virus (TYLCSV) interferes with jasmonate signalling when transgenically expressed in *Arabidopsis* or *Nicotiana benthamiana*. Given that the SCFCOI1 complex is the jasmonate receptor, it would be feasible to speculate that the C2-mediated inhibition of jasmonate responses is likely due to the effect of C2 on this complex. However, transcriptomic analyses show that C2 is not generally affecting the transcriptional response to jasmonates, but specifically suppressing jasmonate-triggered defence responses and secondary metabolism. Consistently with the inhibition of jasmonate-mediated defences, transgenic plants expressing C2 are more susceptible to biotrophic pathogens. Interestingly, we found that C2 from TYLCSV, but not C2 from the curtovirus Beet curly top virus (BCTV), physically interacts with a member of the JAZ family of repressor proteins, and that overexpression of this JAZ protein exerts a negative impact on the infection by begomoviruses. Based on our findings, we propose that C2 from begomoviruses may interfere with the jasmonate response at multiple levels.

Venom allergen-like proteins secreted by plant-parasitic nematodes modulate defense responses mediated by extracellular innate immune receptors

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Plants and animals utilize cell-surface receptors to survey their direct environment for molecular patterns uniquely associated with infections by microbial invaders. Recently, we showed that a venom allergen-like protein Gr-VAP1 from the plant-parasitic nematode *Globodera rostochiensis* targets the papain-like cysteine protease $Rcr3^{pim}$ that functions as a cofactor of the extracellular plant immune receptor Cf-2 in tomato. However, the presence of $Rcr3^{pim}$ alone almost doubles the susceptibility of tomato plants to *G. rostochiensis*, suggesting that Gr-VAP1 modulates the activity of $Rcr3^{pim}$ to enhance the virulence of the nematodes. Although secreted venom allergen-like proteins are highly conserved among plant-parasitic nematodes, their function in parasitism of host plants is largely unknown. Here we demonstrate that venom allergen-like proteins from plant-parasitic nematodes specifically alter defense responses mediated by extracellular innate immune receptors. Knocking-down the expression of venom allergen-like proteins in *G. rostochiensis* strongly reduced the virulence of the nematodes. Ectopic expression of different venom allergen-like proteins from plant-parasitic nematodes in transgenic Arabidopsis plants significantly reduced their basal defense responses to nematodes, multiple other plant pathogens, and the immunogenic peptide flg22 from bacterial flagella. Furthermore, venom allergen-like proteins altered the defense-related programmed cell death activated by multiple extracellular plant immune receptors in leaves of *Nicotiana benthamiana*. We therefore propose that venom allergen-like proteins modulate the activation and/or signaling by extracellular immune receptors.

Tyrosine phosphorylation of a plant PRR is targeted by a *Pseudomonas syringae* effector to suppress plant immunity

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Perception of pathogen-associated molecular patterns (PAMPs) by surface-localised pattern-recognition receptors (PRRs) is a key component of plant innate immunity. Most known plant PRRs are receptor kinases and initiation of PAMP-triggered immunity (PTI) signalling requires phosphorylation of the PRR complex. However, the exact phosphorylation events and their biological roles are still unknown. Tyrosine (Tyr) phosphorylation of plant receptor kinases was only demonstrated recently, but its role in innate immunity has never been reported. Here, we reveal that PRR tyrosine phosphorylation is critical for PTI signalling and immunity. We show that the *Arabidopsis* leucine-rich repeat receptor kinase EFR, which perceives bacterial EF-Tu (or its immunogenic epitope elf18), is phosphorylated on tyrosine residues and that this modification is critical for EFR activation upon ligand binding. A single tyrosine residue of EFR kinase domain is required for EFR activation, downstream responses and immunity to the phytopathogenic bacterium *Pseudomonas syringae*. Successful pathogens must suppress PTI, and many bacteria do so by injecting effector proteins into the plant cell via the type-III secretion system. Consistent with the critical role of PRR tyrosine phosphorylation in initiating PTI signalling, the *P. syringae* type III-secreted effector HopAO1, which is a tyrosine phosphatase, directly targets EFR to inhibit its activation and subsequent immunity. Our results shed light on a novel regulatory mechanism controlling plant immune signalling that is critical for anti-bacterial immunity.

Nematode-induced nucleolar GTP-binding protein is crucial for compatible plant nematode interaction.

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Plant cyst nematodes are common pests of many crops causing substantial losses in agriculture. Their parasitism relays on secretion triggered plant cell developmental reprograming leading to syncytium formation which is a sole food source for growing nematode. To study molecular mechanisms of complex plant nematode interaction the silencing of nematode or host plant genes crucial for pathogenesis is a typical strategy. Currently we focus on NGB gene encoding nucleolar GTP-binding protein. Transcripts of NGB were localized only in young syncytia up to 7 dpi. The regulatory regions of studied gene was cloned upstream the uidA reporter gene and analyzed in tomato and potato roots showing several changes in expression profiles upon infection. Functional analysis was supplemented by the protein subcellular localization which had proven its nucleolar localization in *N. benthamiana* leaves. Silencing NGB genes slightly decreased plant fertility and changed fruit or leaf morphology. This was accompanied by changes in expression of some genes related to auxin and biotic stress signalling. The number of *G. rostochiensis* females was reduced by 57-86% in in vitro tests. The observations of the development and ultrastructure of syncytia induced in transgenic lines revealed retarded growth, electron translucent cytoplasm, smaller vacuoles, reduced number of plastids, mitochondria and ER structures. These results demonstrate that NGB plays an important role in

the development of syncytia and link nematode pathogenesis to ribosome biogenesis. This work was supported by National Science Centre (grants no. NN302-593938 and 2012/07/B/NZ9/02027).

Molecular characterisation of the Prf bacterial recognition complex of tomato.

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The major virulence strategy of phytopathogenic bacteria is to secrete effector proteins into the host cell to target the immune machinery. AvrPto and AvrPtoB are two such effectors from *Pseudomonas syringae*, which disable an overlapping range of kinases in *Arabidopsis* and Tomato. Both effectors target surface-localized receptor-kinases to avoid bacterial recognition. In turn, tomato has evolved an intracellular effector-recognition complex composed of the NB-LRR protein Prf and the Pto kinase. Structural analyses have shown that the most important interaction surface for AvrPto and AvrPtoB is the Pto P+1 loop. AvrPto is an inhibitor of Pto kinase activity, but paradoxically, this kinase activity is a prerequisite for defense activation by AvrPto. Using biochemical approaches we show that disruption of Pto P+1 loop stimulates phosphorylation in trans, which is possible because the Pto/Prf complex is oligomeric. Following P+1 loop disruption and transphosphorylation the Pto/Prf complex dissociate leading to downstream signalling through mitogen-activated protein kinases (MAPKs). Hence, the Pto/Prf complex is a sophisticated molecular trap for effectors and provides an excellent model to study the mechanism of MAPKs activation.

On the search of *Ralstonia solanacearum* AWR5 targets

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Our previous work showed that the *Ralstonia solanacearum* AWRs are a family of effector proteins injected by the pathogen inside plant host cells via the type III secretion system and that they can impact plant defence mechanisms. Our present work focuses on the characterisation of AWR5, which causes hypersensitive response cell death on nonhost *Nicotiana benthamiana*. To characterize AWR5 function we use two approaches: a) Gene expression profiling using heterologous expression of AWR5 in the yeast *Saccharomyces cerevisiae*, where it causes growth inhibition, and b) Proteomic analysis of AWR5 binding partners in *N. benthamiana*. Expression of AWR5 in yeast has revealed a gene expression profile reminiscent of TOR complex hyperactivation: up-regulation of genes involved in ribosome biogenesis or rRNA processing and down-regulation of genes involved in the metabolism of nitrogen. We are currently investigating the mechanism(s) underpinning these AWR5-dependent changes in gene expression. In order to identify AWR5 targets in *N. benthamiana* we are setting up a novel semi-in vivo approach that may also help characterizing other effector-target interactions in diverse plant patho-systems.

Plant response to biocontrol strategies

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Several rhizosphere bacterial strains were selected based on their biocontrol activity against different phytopathogenic fungi, and preserved in the microbial collection of the Research - Development Institute for Plant Protection (RDIPP). Most of the selected strains belong to *Bacillus* species and are involved in suppressing soil borne phytopathogenic fungi. The selection of biocontrol bacteria was based mainly on their biological traits to produce metabolites with antifungal activity and their ability to compete plant pathogens for nutrients and niche. However, the induced systemic resistance (ISR) in plants based on the use of biocontrol bacteria is taken into consideration for further studies. Our studies on ISR mechanisms, initiated in collaboration with the Faculty of Biotechnology from USAMV Bucharest, were focused mainly on the activation of phenylalanine ammonia-lyase gene in tomato plants inoculated with biocontrol bacteria.
The activation of ISR in plants could be an effective and economical way to suppress plant pathogenic attack. Therefore, ISR could be a rapid solution in suppressing new races of pathogens or it could reduce the time and costs required to achieve plant cultivars resistant to different phytopathogenic infections.

Functional analysis of the TALome of an African *Xanthomonas oryzae pv. oryzae* strain reveals a new susceptibility gene candidate for bacterial leaf blight

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Bacterial plant pathogenic Xanthomonads translocate 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 (from 9 to 16) in their genome. While one or two encode major virulence factors, the relative contribution of each of the other members to *Xoo* pathogenicity remains unclear. To address that question, we aimed at performing a systematic functional analysis of the complete TALome of *Xoo* strain MAI1, which originates from Mali and contains a reduced set of *TAL* genes as compared to strains from Asia. We identified 9 different *TAL* genes from a cosmid genomic DNA library and subcloned them into an expression vector suitable for functional analysis. TAL effector-deficient *Xoo* strain X11-5A carrying each single *TAL* gene from MAI1 were assessed for gain of virulence on susceptible rice. Interestingly, at least three *TAL* genes were found to provide increased virulence to X11-5A two of which are known to target the *S* susceptibility gene *SWEET14.* Using a program for TAL target prediction, a third TAL effector was suggested to target a new susceptibility gene candidate. Our most recent data on the functional analysis of this new TAL effector and its target will be presented.

Effector evolution and diversification (WG3)

Oral Presentations

Thierry Rouxel (INRA-Grignon, FR)
Evolutionary dynamics of avirulence genes in *Leptosphaeria maculans*.

Genome sequencing and expression profiling of emerging strains of P. infestans
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In 2005, a clonal lineage of the potato late blight pathogen *P. infestans*, genotype blue 13_A2, was identified in the UK. The increased aggressiveness of this genotype and its virulence on several resistant potato varieties led to it becoming the most prevalent genotype in UK within just a few years. In the US, genotype US22 caused a massive epidemic on tomato during the summers of 2009 and 2010 in Eastern USA and Canada. To fully characterize the genetic basis for the success of these genotypes, we performed genome sequence and gene expression analysis. We discovered that the blue 13 strain, 06_3928A, exhibits significant genetic and gene expression polymorphisms. In addition, we found that 06_3928A exhibits a sustained gene induction pattern and an extended biotrophic growth phase during infection. Interestingly, 06_3928A carries intact and in planta induced Avrblb1, Avrblb2 and Avrvnt1 genes and is avirulent on potato lines that carry Rpi-blb1, Rpi-blb2 and/or Rpi-vnt1.1 genes. For the US22 strain, P17777, although this genotype is more aggressive on tomato when compared to the reference strain T30-4, our gene expression analysis showed that the majority of genes in P17777 were induced in both potato and tomato. This result indicates that the P17777 may be "host blind" and that without management US22 could also become a threat to potato. Our findings illustrate how pathogen genome analysis can assist with the management of destructive plant disease epidemics.

Insights in the evolution of plant parasitism within the phylum Nematoda based on neutral and pathogenicity-related genes.
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Plant parasitism has arisen at least four times independently within the phylum Nematoda. As a starting point to reconstruct evolution of parasitism within this ubiquitous and diverse group of animals, we use (nearly full length) small subunit ribosomal DNA (SSU rDNA) sequences (≈ 1,700 bp). Currently this phylum-wide framework encompasses over 2,500 taxa, and phylogenetic analysis reveals relationships within and among these four major lineages. Remarkably, SSU rDNA - known as a conserved gene - shows accelerated evolution in case of branches dominated by taxa with short life cycles, and/or animal or plant-parasitic life styles.

One of the practical implications of this characteristic is the availability of unique DNA motifs within this gene that allows for relatively straightforward high-throughput detection of plant-parasitic nematode species in complex (= environmental) samples. Currently over 30 tests have been developed including most of the high-impact root knot, cyst and lesion nematode species attacking both Solanaceous and/or cereal crops.
In parallel we investigated relationships within individual lineages of plant-parasitic nematodes with regard to non-neutral, plant pathogenicity-related genes, and by doing so we tried to find clues about the role of horizontal gene transfer in the transition from bacterivores (ancestral state) towards fungivores and facultative & obligate plant parasites (most derived state). Analysis of distribution and diversity of cell wall-degrading enzymes suggest for a very early acquisition, and subsequent diversification, and it would be highly interesting to look for related patterns in effector evolution.

Evolution of the pan-secretome among lineages of Magnaporthe oryzae attacking different host-plants.

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Over the past decade, great advances have been made in the understanding of the role of fungal effectors in the infectious process, especially small secreted proteins (SSP). NGS offer powerful tools to study at the genomic scale how deep are SSP involved in the adaptation of fungal populations to their hosts. We addressed this question in the fungus *Magnaporthe oryzae*, the agent of blast on rice and other Poaceae. In this species, isolated genetic lineages specifically attack different hosts. We sequenced 8 strains of *M. oryzae* from lineages pathogenic to different Poacees (5 strains attacking rice *Oryza sativa*, 1 attacking wheat *Triticum* sp., 1 attacking foxtail millet *Setaria* sp., 1 attacking *Eleusine* spp.), and 1 strain of the sister species *M. grisea* (attacking *Digitaria* spp). The 9 genomes have been sequenced using NGS (454 and Illumina) and assembled by the Genoscope (Evry, France). We included the public reference genome *M. oryzae* 70-15 in our analyses. We carried out gene annotation, gene families prediction and transposable elements characterization.
Here we present the characterization of SSP repertoires in the 10 genomes, established using predictors of peptide signals (SignalP), transmembrane domains (TMHMM), GPI anchors (PrediGPI) and subcellular location assignment (TargetP). These lists were curated using two complementary approaches: systematic tBlastn searches of the SSP predicted in each genome against the nine genomic databases of the project (including its own), and gene mining through the RNAseq analysis of the in planta transcriptome of one of the strain. We will compare these lists with orthology predictions to analyze the core-secretome and the dynamics of gains/losses/duplications of SSP in the different lineages. We will also address the question of co-localization of SSP with transposable elements. Finally we will search for signatures of adaptive evolution in SSP.

Extensive Chromosomal Reshuffling Drives Evolution of Virulence in an Asexual Pathogen.

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Sexual recombination drives genetic diversity in eukaryotic genomes and fosters adaptation to novel environmental challenges. Although strictly asexual microorganisms are often considered as evolutionary dead ends, they comprise many devastating plant pathogens. Presently, it remains unknown how such asexual pathogens generate the genetic variation that is required for quick adaptation and evolution in the arms race with their hosts. Here, we show that extensive chromosomal rearrangements in the strictly asexual plant pathogenic fungus *Verticillium dahliae* establish highly dynamic lineage-specific (LS) genomic regions that act as a source for genetic variation to mediate aggressiveness. We show that such LS regions are greatly enriched for in planta-expressed effector genes encoding secreted proteins that enable host colonization. The LS regions occur at the flanks of chromosomal breakpoints and are enriched for retrotransposons and other repetitive sequence elements. Our results suggest that asexual pathogens may evolve by prompting chromosomal rearrangements, enabling rapid development of novel effector genes. Likely, chromosomal reshuffling can act as a general mechanism for adaptation in asexually propagating organisms.

David Cooke (James Hutton Institute, UK)

Tracking effector diversity in *Phytophthora infestans* populations.

Inventory and evolution of Type III effector proteins in the *Ralstonia solanacearum* species complex

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Ralstonia solanacearum is a soil-borne beta-proteobacterium that causes bacterial wilt disease in many food crops and specifically solanaceaous crops. It is a major problem for agriculture in intertropical regions. *R. solanacearum* is a heterogeneous species, both phenotypically and genetically, and is considered as a species complex. Pathogenicity of *R. solanacearum* relies on the Type 3 secretion system that injects Type 3 effector (T3E) proteins into plant cells. T3E collectively perturb host cell processes and modulate plant immunity to enable bacterial infection.
We provide the catalogue of T3E in the *R. solanacearum* species complex, as well as candidates in newly sequenced strains. 95 T3E orthologous groups were defined on phylogenetic bases and ordered using a uniform nomenclature. This curated T3E catalog is available on a public website and a bioinformatic pipeline has been designed to rapidly predict T3E genes in newly sequenced strains. Systematical analyses were performed todetect lateral T3E gene transfer events and identify T3E genes under positive selection. Our analyses also pinpoint the RipF translocon proteins as major discriminating determinants among the phylogenetic lineages. Establishment of T3E repertoires in strains representatives of the *R. solanacearum* biodiversity allowed determining a set of 22 T3E present in all the strains but provided no clues on host specificity determinants. The definition of a standardized nomenclature and the optimization of predictive tools will pave the way to understanding how variation of these repertoires is correlated to the diversification of this species complex and how they contribute to the different strain pathotypes.

The structure and evolution of barley powdery mildew effector candidates

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The powdery mildew fungus, *Blumeria graminis f.sp. hordei* (Bgh) is a recently sequenced obligate biotrophic pathogen of barley. In addition to being a significant agricultural pathogen, it also serves as a model for studies on powdery mildew fungi and other obligate biotrophic pathogens. I will present a comprehensive survey of 491 Candidates for Secreted Effector Proteins (CSEPs) representing more than 7% of the protein coding genes found in the Bgh genome. Based on sequence homologies, we clustered the CSEPs into families of paralogs and show that CSEP genes have duplicated in the genome, most likely due to unequal crossing over during evolution, which has caused them to cluster in the genome. Within many of these families, we find strong evidence for positive selection for diversity. When we mapped the amino acid residues under positive selection on 3D structural models, they were usually predicted to be exposed on the protein surface, and thus possibly involved in protein interactions. Expression studies show that the CSEPs preferentially are expressed in haustoria. Many CSEPs across different families appear to be related to microbial RNases, and we propose that a large proportion of the CSEPs have evolved from an ancestral microbial RNase. We speculate that these RNases may have been an ideal starting material for building up an effector arsenal. Our data fit well with a model for CSEP evolution driven by selection for gene duplications and for amino acid chances resulting in a large diversity allowing the fungus to yield a highly diverse palette of effector functions.

Effector evolution and diversification (WG3)

Poster Presentations

Evolution of great diversity in a Central European population of *Blumeria graminis f. sp. hordei.*

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Powdery mildew caused by *Blumeria graminis f. sp. hordei* is one of the commonest diseases of barley in Europe and in other parts of the world. The pathogen can change rapidly in virulence and virulence associations, and such changes based on specific resistances of the host may overcome resistance in cultivated varieties and result in severe epidemics. To monitor changes in the pathogen populations, isolates obtained by means of a mobile version of a jet spore

sampler collected in the Czech Republic were tested on 57 barley differential varieties to identify pathotypes of *B. graminis f. sp. hordei*. In 2011, 150 isolates and in 2012, 144 isolates were evaluated and in total 292 pathotypes were found. Virulence frequencies to specific resistance genes in differential varieties and virulence complexity were also monitored. In Central Europe many bred varieties with specific resistances have been used and new pathogen immigrants with emerging virulences originating in the rest of Europe often occur here. Therefore, it is not surprising that the Czech population displays great diversity which is reflected in its rich pathotype composition.

Genome analysis of *Vertiillium tricorpus* – example of mild vascular pathogen

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Verticillium wilts are vascular diseases caused by *Verticillium* spp, causing crop losses that amount to billions of dollars every year. Based on its economic impact, *Verticillium dahliae* is the most detrimental, infecting hundreds of host species worldwide. *Verticillium tricorpus* is closely related to *Verticillium dahliae,* but has a much narrower host range and is considered as a mild pathogen as it causes mild or no symptoms on tomato, whereas *V. dahliae* can cause strong stunting symptoms. In order to understand this differential aggressiveness on tomato, a comparative genomics study between *V. tricorpus* and *V. dahliae* was initiated. The genome of *V. dahliae* has been sequenced previously, whereas a genome sequence of *V. tricorpus* was not available. In this study, the draft genome of *V. tricorpus* is determined. The genome was sequenced using Illumina technology. In order to obtain an optimal assembly, we compared different assembly tools in combination with various error correction software packages. The best assembly, with respect to the highest N50, least amount of scaffolds, and least amount of gaps, was generated by an assembly software package which is named the A5 pipeline. Additionally, we used long sequence reads generated with PacBio sequencing to improve the draft genome assembly. The final assembly consists of 61 scaffolds and 347 gaps. Genes were annotated using RNAseq analysis and the Maker2 software package, which resulted in 12,442 genes. Among these genes, 1,353 were predicted to encode secreted proteins. In conclusion, we assembled and annotated the draft genome of *V. tricorpus* using a hybrid assembly method (Illumina- and PacBio reads) and RNAseq data. This provides a starting point for future comparative genomics studies.

Comparative pathogenomics of cereals powdery mildew disease (*Blumeria graminis*)

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Powdery mildew (*Blumeria graminis*) is a major cereals pathogen causing consistent yield loss in many world regions. *B. graminis* is a obligate biotrophic fungus and host specific pathogen that generally can infect only one plant species. Up to date 8, formae speciales that infect different plants have been described. The genomes of barley (*B.g. hordei*) and wheat (*B.g. tritici*) powdery mildew have been sequenced recently. Both contain 500-600 putative effector genes. We found that different wheat mildew isolates differ mainly in the presence/absence of such candidates. Triticale (xTriticosecale) is hybrid between wheat (used as male parent) and rye (used as female parent) that has been commercialized since the end of the 1960s. It is an

artificial allopolyploid, and many lines with different ploidy levels have been created from different wheat species. Powdery mildew has become pathogen of this new host only in the last ten years. This new forma speciales has evolved from the wheat powdery mildew through a host range expansion. In order to understand how powdery mildew could breakdown triticale resistance simultaneously in different European countries we are sequencing with next generation technologies multiple isolates of triticale and rye (*B.g. secalis*) powdery mildew in addition to wheat powdery mildew data that we already have. In order to sample a large diversity more than 70 triticale powdery mildew isolates were collected in 5 different sites across Switzerland. Further isolates have been requested from other labs. One specific objective of our study is to identify effector candidates in the triticale powdery mildew genome and compare then to effector sets of other formae speciales such as wheat and rye powdery mildew. Our study aims at unravelling the genetic basis of effector mediated host-pathogens coevolution.

Molecular dynamics of the RBP-1 effector gene family in the potato cyst nematode *Globodera pallida*

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The potato cyst nematode species *Globodera rostochiensis* and *G. pallida* have a narrow host range which is restricted to Solanaceous species, including tomato, potato and egg plant. Upon invasion of the roots, they transform cells near the vascular cylinder into a permanent feeding site on which they fully depend for their development. The host cell modifications are most likely induced by a complex mixture of proteins which are secreted by the nematode during early stages of infection. However, host plants have evolved strategies to counterbalance the establishment of such a feeding relationship, including effector triggered immunity. In return, virulent plant parasitic nematodes have developed means to overcome host resistance. Recently, we demonstrated that members of the SPRYSEC family from the potato cyst nematode *G. rostochiensis* are able to suppresses CC-NB-LRR mediated cell death and disease resistance in plants. *Globodera pallida* RBP-1 encodes a related secretory protein with a SPRY domain, which is specifically recognized by the potato immune receptor Gpa2. All RBP-1 variants found in the avirulent population of *G.pallida* (D383) elicit a Gpa2-dependent HR, while a virulent population (Rookmaker) possessed two variants which evade Gpa2 recognition due to a single DNA polymorphism. This is the result of diverifying selection, which has contributed to the expansion of this effector gene family in potato cyst nematodes. Here, we present novel data on the complexity and sequence diversity of the RBP-1 gene family, illustrating the molecular dynamics underlying the co-evolution between *G. pallida* and Solanaceous plant species. This will contribute to our understanding of nematode (a)virulence and the development of broadspectrum nematode resistance.

R genes and host targets for resistance breeding and engineering (WG4)

Oral Presentations

Exploiting pathogen effectors in breeding for disease resistance
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Cultivated potato (*Solanum tuberosum*) severely suffers from the devastating late blight disease, which is caused by *Phytophthora infestans*. This oomycete pathogen effector proteins during infection of potato plants. Part of its effector repertoire is represented by RXLR effectors that can traffic inside plant cells. Some of these RXLR effectors represent avirulence (AVR) proteins and these can be recognized by intracellular immune receptors of the potato. Based on this rationale, we have initiated an effectoromics strategy to identify immune receptors from wild *Solanum* species. We have generated a genome-wide infection-ready library of RXLR effectors of *P. infestans* RXLR effectors and used these effectors to identify resistance (*R*) genes in an extensive collection of wild *Solanum* species and breeding material. This has proven effective and complementary to classical breeding approaches; more than ten R-AVR pairs are already available that can be immediately exploited to accelerate and improve late blight resistance breeding. Recently, we have expanded the cytoplasmic R-AVR-based line of defense with studies on apoplastic immunity, which has generally a broader spectrum and is based on recognition of conserved proteins of pathogens. Screens with apoplastic effectors that have features of pathogen-associated molecular patterns (PAMP) has led to the identification of new types of receptors in potato. We have isolated a potato pattern recognition receptor ELR that senses elicitins, which are considered PAMPs of oomycetes. In transgenic potatoes, *ELR* confers enhanced resistance to late blight. Our strategy is to combine multiple layers of immunity and achieve effective and durable resistance against late blight in potato.

Lectin receptor kinases; novel leads for disease resistance in Solanaceous crops.
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Plant receptor kinases play pivotal roles in sensing invading pathogens and initiating host defense responses. In recent years, we identified a novel type of resistance in *Arabidopsis* to *Phytophthora* pathogens. This resistance is mediated by the cell surface receptor LecRK-I.9, which belongs to the family of L-type lectin receptor kinases (LecRKs). Expression of LecRK-I.9 as transgene in potato and *Nicotiana benthamiana* increases resistance to *P. infestans*, demonstrating that the resistance function of LecRK-I.9 is maintained after interfamily transfer. Next to LecRK-I.9, multiple other *Arabidopsis* LecRKs have been pinpointed to function in pathogen resistance. Bioinformatic analysis aimed at finding homologs of the *Arabidopsis* LecRKs in Solanaceous plant genomes resulted in the identification of multiple LecRKs of potato, tomato and *N. benthamiana* (SolLecRKs). Multiple of these SolLecRKs have been further investigated for their functionality in *Phytophthora* resistance. Gene silencing of several SolLecRKs resulted in enhanced disease susceptibility, indicating that LecRKs play similar roles in disease resistance in Solanaceous plants. These results justify exploitation of LecRKs as novel leads for disease resistance in Solanaceous crops.

The *Magnaporthe oryzae* effectors AvrCO39 and Avr-Pia are recognized by the rice Nucleotide Binding-Leucine rich repeat (NB-LRR) protein RGA5 through direct interaction

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Plant immunity strongly relies on direct or indirect recognition of pathogen effectors by plant resistance (R) proteins. This recognition activates disease-resistance signaling pathways leading to the inhibition of pathogen growth and the induction of a localized programmed cell death called the hypersensitive response (HR). To gain a better understanding of the molecular mechanisms governing effector recognition in plants, we study two translocated effectors from the rice blast fungus *Magnaporthe oryzae*: Avr-Pia and AvrCO39. Our work shows that both sequence-unrelated effectors are recognized by the same duo of rice NB-LRR proteins, RGA4 and RGA5. RGA4 and RGA5 genes are located next to each other on rice chromosome 11 and are both necessary to confer resistance to *M. oryzae* strains expressing either Avr-Pia or AvrCO39. Interestingly, RGA5 transcripts are alternatively spliced leading to the production of two protein variants termed RGA5-A and RGA5-B. Yeast two hybrid analysis revealed that Avr-Pia physically and specifically interacts with RGA5-A via a small RGA5-A specific domain whereas AvrCO39 interacts with RGA5-B via another small RGA5-B specific domain. This suggests that RGA5-A and RGA5-B act as receptors mediating specific recognition of the effectors by direct binding while RGA4 might act as a signaling component activating downstream resistance pathways. Furthermore, these results indicate that alternative splicing might be a mechanism contributing to the evolution and diversification of plant R-gene repertoires. Recent advance in the confirmation of RGA5-A and -B recognition specificities and in the validation of the observed interactions will be presented.

Using TALENs for plant genome engineering.

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The DNA-binding domain of transcription activator-like effectors (TALEs) from Gram-negative plant-pathogenic *Xanthomonas* bacteria has become an important tool for the programmable and specific targeting of DNA. TALE proteins bind to DNA via near-identical tandem repeats of 34 amino acids. Each repeat recognizes one base in the target DNA sequence via repeat-variable diresidues (RVDs). The simple and modular repeat architecture allows rearrangement of TALE repeats to generate artificial TALEs with virtually any tailored DNA-binding specificity. Highly specific genome-editing TALE nucleases (TALENs) can be engineered for targeted mutagenesis in plants and a wide variety of other eukaryotic organisms. We will introduce the technology and demonstrate TALEN activities in vitro and in vivo. We modified target DNA sequences in plant cells using TALENs in a transient *Nicotiana benthamiana* reporter system and in rice calli. TALEs are exceptional tools for plant genome engineering and open the possibility to generate pathogen-resistant non-GMO plants.

Integration of genetics and RNA-Seq approaches to dissect a durable blast resistance in rice.

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The hemibitroph *Magnaporthe oryzae* is the most important rice fungal pathogen causing worldwide annual yield losses of about 10-30%. Gigante Vercelli (GV) and Vialone Nano (VN) are two old temperate *japonica* Italian rice cultivars with contrasting response to blast infection: GV displaying durable and broad resistance and VN highly susceptible. A GV x VN segregating population, used to develop an SSR-based genetic map, led to the identification of two loci, localized to the long arms of chromosomes 1 and 4, responsible for blast resistance in GV. The unconscious pyramiding of two blast resistance genes was therefore causal of the observed GV durable resistance. RNA-seq was then used to dissect the early molecular processes deployed during the resistance response of GV at 24 h after blast inoculation. Differential gene expression analysis identified 726 and 699 up regulated genes in response to infection in GV and VN, respectively. GV exhibited a dramatic up-regulation of defense genes including genes encoding diterpene phytoalexin biosynthetic enzymes, flavincontaining monooxygenase and genes involved in the early steps of defence perception-signalling, that included chitin oligosaccharides sensing factors, wall associated kinases, MAPK cascades and WRKY transcription factors. Genes homologs to the major class of the plant disease resistance genes (NB-LRR) and displaying expression patterns consistent with a potential role as GV-specific functional resistance gene(s) were also identified. When NB-LRR differentially regulated among GV and VN were localized on the Nipponbare reference genome, a co-localization of four of them was observed with the two loci responsible for blast resistance in GV. The integration of genetics and transcriptomics studies has therefore provided candidates for the GV durable resistance. The ongoing de-novo assembly of the GV genome will allow a better definition of the positional relationships between the candidates and the blast resistance loci.

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Overview of tomato candidate pathogen recognition genes

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Tomato (*Solanum lycopersicum*) has served as an important model system for genetics and genomic studies. In this study, a complete set of pathogen recognition genes was identified in the genome sequence of tomato. These putative R genes were characterized with respect to structural diversity, phylogenetic relationships and chromosomal distribution, and compared with R genes that have now been cloned from different plant species. We found 769 coding sequences, including TNL proteins, CNL proteins, RLP proteins RLK proteins as well as other different domains arrangements. The physical organization of R-genes revealed a large number of mixed clusters. In some instances, duplicated genes within clusters were identified. These

duplication events provide a basis to understand lineage-specific R-gene development in an evolutionary context, as most of them have a recent evolutionary origin. Analysis of R-gene clusters associated with documented resistance function allowed identification of adaptive divergence events and reconstruction of the evolution history of these loci. Differences in candidate pathogen recognition gene number and organization were found between tomato and potato. Most candidate pathogen recognition gene orthologues were distributed at less than perfectly matching positions, suggesting an ongoing lineage-specific rearrangement. Our findings can improve understanding of the mechanisms of molecular adaptive selection at *Solanum* R loci

Study of durable disease resistance *Lr34* wheat gene into heterologous grass species

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Lr34 is one of most important resistance genes in wheat, providing durable resistance against multiple biotrophic fungal pathogens. *Lr34* expression is strongest in flag leaves of adult wheat plants and is associated with the morphological trait leaf tip necrosis (LTN). It encodes for an ATP-Binding Cassette (ABC) transporter of the PDR subfamily and occurs in two protein variants which are either associated with resistance or with susceptibility: the resistant *LR34* protein differs by only two amino acid polymorphism from the susceptible version. The objective of our research is to understand the mechanisms of *Lr34*-mediated durable resistance.
The genetic complexity of wheat has made accessibility to genome information and functional gene analysis difficult until now. Therefore, we established a heterologous expression system which would allow more insights into the molecular mechanism(s) underlying *Lr34* gene function. For this, we transferred Lr34 into barley and rice. In barley we found that wheat *Lr34* retained its function after the transfer and the expression of the genomic *Lr34* resistant sequence induced a premature leaf tip necrosis in seedlings. *Lr34* protected barley against barley leaf rust and barley powdery mildew pathogens.
The heterologous expression of *Lr34* revealed that its underlying mechanism is conserved amongst wheat and barley. This raises the possibility that the *Lr34* resistance gene can protect other agriculturally important crops against their host-specific pathogens.
Publications
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R genes and host targets for resistance breeding and engineering (WG4)

Poster Presentations

Maize association population for fungal diseases resistance studies

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Portugal is well known for the still cultivated numerous maize landraces adapted to specific regional growing conditions and farmers needs. Preliminary field characterization of this national plant germplasm has identified the existence of high variability for resistance to diseases (*Puccinia sp., Ustilago maydis* and *Fusarium sp*.). Disease incidence varied, in the case of *U. maydis* and *Fusarium sp*. infection, from complete absence of symptoms till high susceptibility. Nevertheless, on average, a low disease incidence was observed. However, in the case of the rust infection all the material was partially or highly susceptible, with no cases of complete resistance.
We have been developing research on a highly variable collection of maize inbred lines many of each selected from these traditional Portuguese landraces. This collection is presently being genotyped with the Illumina MaizeSNP50 BeadChip array. This plant germplasm/high throughput genotyping tool set represents a very interesting base system for future broad-spectrum disease resistance association studies, possibly allowing us to identify allelic variants for the out coming FA1208 action effector's targets.

Wheat Lr34 provides multi-pathogen resistance in barley.

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Lr34 of wheat is one of the few durable resistance genes in crop plants and is used in breeding for over 100 years. The gene, encoding for an ABC transporter, was first described to provide quantitative resistance to wheat leaf rust. In addition, partial resistance to stripe rust, stem rust and powdery mildew was observed. An additional phenotype of Lr34 is leaf tip necrosis (LTN), a senescence-like process that affects flag leaves of adult wheat plants. Lr34-mediated resistance is unique to wheat and is not found in closely related grasses such as barley. We therefore transformed barley with genomic Lr34. Transgenic barley became more resistant to barley leaf rust (*Puccinia hordei*) and barley powdery mildew (*Blumeria graminis f.sp. hordei*). In contrast to wheat, resistance was effective already in seedlings. However, resistance was accompanied by strong leaf necrosis resulting in reduced plant vigour and seed sets. The aim of this project is to engineer barley plants with adequate Lr34 resistance while plant growth and yield production are not affected. We assume that the severe phenotype is due to miss-regulated gene expression, posttranslational regulation or protein stability. Therefore we will fine-tune Lr34 expression and protein stability in barley.

Several Wall-Associated kinases play individual and contrasted roles in quantitative resistance to rice blast

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Receptor-like kinases are well-known to play key roles in disease resistance. Among them, the Wall-associated kinases (WAKs) have been shown to be involved in fungal disease resistance in *Arabidopsis* and rice although the direct requirement of these genes in resistance is not established. Here we show, using rice loss-of-function mutants of four OsWAK genes that individual OsWAKs are required for quantitative resistance to the fungal pathogen responsible for rice blast, *Magnaporthe oryzae*. While the transcription of the majority of the WAK genes is up-regulated during infection, OsWAK112d is down-regulated. In addition, we show that most of the very early transcriptional regulations of the rice WAK genes are partially under the control of the chitin receptor CEBiP and the OsWRKY28 transcription factor. While OsWAK14, OsWAK91 and OsWAK92 positively regulate quantitative resistance, OsWAK112d is negatively regulating resistance to blast fungus. This is the first report of a WAK gene acting as a negative regulator of disease resistance. Y2H and bi-molecular fluorescence complementation experiments indicate that WAK proteins form a complex in the plant membrane through interaction with their extracellular domains. Thus OsWAKs are required for the establishment of fine-tuned quantitative resistance in rice.

PRGdb 2:0: a community-based database for the analysis of R-genes in plants

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The exponential accumulation of information in in plant-pathogen interaction require storage and management of data to extract important meanings and to connect them with a variety of resources that cater to the needs of biological research. The Plant Resistance Genes database, lauched in 2009, (http://www.prgdb.org) is the first bioinformatic resource that provide a comprehensive overview of resistance genes (R-genes) in plants. In the last years, PRGdb has grown more than 6 fold to recently include annotation derived from important plant genome sequencing projects. Our database offer a range of querying and mining tools to explore the data and enable discovery. The development of the PRG platform represents an important starting point to conduct various experimental tasks. The inferred cross-link between genomic and phenotypic information allows access to a large body of information to find answers to several biological questions. The current release 2.0 hosts biological information on a set of 112 known and 104,310 putative R-genes present in 233 plant species and conferring resistance to 122 different pathogens. It becomes a community database for plant scientists who could in turn contribute to this public resource through a WIKI-like system. Moreover, the website has been completely redesigned with the implementation of Semantic MediaWiki technologies, which makes this repository freely accessed and easily edited by any scientists. The implementation performed is aimed to create a source for easily data sharing and to centralize users contributions. We encourage plant biologist experts to join our annotation effort and share their knowledge on disease resistance with the rest of the scientific community. PRGdb is intended as long-term, consistently maintained, community resources.

Determination of the Functions of SRP72 and CAS Barley Homologues Against Powdery Mildew Disease Resistance in Barley

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The barley Mla1 gene is one of the genes that are responsible for the resistance against the Powdery Mildew fungus *Blumeria graminis f. sp. hordei* (Bgh). The main goal in this study is to understand the signaling events after the Mla1-AvrMla1 recognition and the death of the plant cell. For this purpose the genes SRP72 (Signal Recognition Particle 72) and CAS (Cellular Apoptosis Susceptibility) are silenced. The transcripts of these gene products were detected to be induced in Mla1 mediated disease resistance in barley and they were likely to have roles in the conserved pathway which is activated by R-Avr recognition. These proteins have functions in literature that may relate them to the disease resistance in plants that is why their functions are determined by Virus Induced Gene Silencing (VIGS).
After silencing of the plant homologues of SRP72 and CAS in barley cultivar "Pallas-01" (which has the Mla1 for the resistance against powdery mildew disease of barley), change in resistance response is observed. Up to this date these proteins that are in study are not evaluated in terms of the plant disease resistance.
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Towards synthetic disease resistance genes

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Pathogens have developed ways to modulate host processes and immunity enabling parasitic infection. In turn, plants have deployed different defence mechanisms, reflecting a co-evolutionary arms race between plants and pathogens. Some plant resistance mechanisms follow the 'gene-for-gene' model, which explains the relationship between pathogen races and host cultivars by the interaction between an avirulence effector (Avr) gene in the pathogen race and an R gene in the cultivar. Effector proteins (AVR) with avirulence activities are recognised by their cognate resistance protein (R) in the plant. Understanding the mechanism of recognition of AVR effectors by R immunoreceptors should prove helpful in managing and improving R-mediated resistance. The ascomycete *Fusarium oxysporum f. sp. lycopersici* (FOL), causal agent of wilt in tomato, is responsible for significant losses worldwide. Three races of FOL have been reported whose relationship with tomato cultivars is explained by the 'gene-for-gene theory'. I, I2 and I3 are the R genes in tomato that correspond to FOL's avirulence effector genes Avr1, Avr2 and Avr3, respectively. Our goal is to create synthetic R mutant genes with expanded pathogen recognition specificities, as a way to develop broad-spectrum solutions to plant pathogens sharing the same effector families. We are transferring previously characterized R gene mutations that confer expanded recognition to effectors of another important pathogen, the oomycete *Phytophthora infestans*, into I2, to assess whether these mutations also confer expanded activity in I2. Also, we plan to swap domains between orthologous R proteins with different specificities so as to identify regions conferring AVR recognition specificity. These results could lead to new insights into the molecular interactions underlying pathogen perception by plants, building up our knowledge in both basic and applied plant pathology.

Arabinogalactan proteins of border cells: at the frontier between root and microbes.
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The plant root system is a vital organ that serves to anchor the plant in the soil and to absorb nutrients and water. At the root tip, the root cap produces a population of living, metabolically active cells that are released into the rhizosphere as border cells or border-like cells (Hawes et al., 2000; Vicré et al., 2005). Root border cells are defined as cells that originate from the root cap meristematic cells and that disperse individually into suspension when root tips are placed in water (Hawes et al., 2000). Due to their particular position at the interface between root and soil, root border cells play a major role in the interaction with soilborne microorganisms (Driouich et al., 2007; 2010). Our objective is to unravel the role of border cells and border-like cells in plant-pathogen interaction taking advantage of the plant model Arabidopsis thaliana, and some economically-important crop such as pea and potato. We found that cell walls of both border cells and border-like cells were enriched in arabinogalactan proteins or "AGPs" (Durand et al., 2009; Cannesan et al., 2012). AGPs are plant proteoglycans heavily glycosylated, located at the periphery of the cell surface and in the rhizosphere, which are known to play several functions in root biology, including plant microbe interactions (Nguema-Ona et al., 2013). Our findings highlight a novel role for these proteoglycans in root defense against pathogens (Cannesan et al., 2012).
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Verticillium dahliae is an economically important pathogen of a range of horticultural crops. Resistance to *V. dahliae* is predominantly additive and oligogenic in the octoploid cultivated strawberry. Five QTL have been identified using field phenotyping in highly diseased soils. These QTL are robust across years, though do not fully explain the variation seen in mapping lines, indicating that there may be as yet unidentified QTL in the mapping population, or more complex genetic interactions between loci. The process of QTL validation is now underway and pathogenicity data is being gathered for single isolates in order to identify whether there is any cryptic structure in *V. dahliae* populations. Isolates of *V. dahliae* with differences in pathogenicity have been sequenced using longer Illumina reads and a preliminary analysis of data is presented.

Nuclear import of N receptor in tobacco is regulated by posttranslational modification of SGT1 protein
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Resistance to Tobacco Mosaic Virus (TMV) is mediated by the tobacco N protein, which belongs to the TIR-NB-LRR (Toll-Interleukin Receptor; Nucleotide-Binding; Leucine-Rich Repeat) class of plant resistance (R) proteins. N interacts with helicase domain of TMV replicase and shortly after its recognition activates salicylic acid-induced protein kinase (SIPK), which phosphorylates multiple substrates in plant cell. We have recently shown that one of the specific SIPK targets is SGT1 protein, that in concert with HSP90 and RAR1 stabilizes N and maintains it in an inactive but signaling-competent state. Substitutions within the phosphorylation site of SGT1 impair N-mediated resistance to TMV. Interestingly, phospho-mimic mutation leads also to an increased proportion of cells displaying SGT1 nuclear accumulation. Since forced nuclear localization of SGT1 causes N to be confined to nuclei we proposed a model, in which phosphorylation of SGT1 maintains balanced nucleocytoplasmic partitioning of N, that is necessary for its function. SGT1 is able to relocate N protein in only one direction, towards the nucleus. However, our preliminary data show, that co-expression of SGT1 fused to nuclear export signal together with only one of two alternative splice forms of N results in an export of the encoded protein to the cytoplasm. This suggests that N variants form heterooligomers that affect localization of the N receptor complex within plant cell. We are currently testing this hypothesis.

Accessions of *Oryza barthii* confer resistance to *Xanthomonas oryzae pv. oryzae* due to a deletion in the major susceptibility gene SWEET14

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Xanthomonas oryzae pv. Oryzae (Xoo) is the causal agent of Bacterial Leaf Blight (BLB) of rice, an emergent disease in Africa. To promote growth in planta and leaf vascular tissues colonization, Xoo secretes TAL (« Transcription activator-like ») effectors via the type 3 secretion system. TAL proteins act in the host cell as bona fide transcription factors and recognize specific sequences in the promoter region of host target genes. Among them is the susceptibility S gene SWEET14 which encodes a sucrose efflux transporter and is essential for disease development. OsSWEET14 is targeted at distinct boxes by four TAL effectors belonging to strains of different lineages and geographic origins. The observed evolutionary convergence for the induction of OsSWEET14 reflects its crucial role as a determinant of rice susceptibility to *Xanthomonas*. To search for potential unresponsive allelic variants, the SWEET14 promoter region of sixty accessions from phylogenetically distant *Oryza* species was sequenced. Polymorphism analysis demonstrated a high conservation of the DNA boxes targeted by TalC, Tal5, Pthxo3 and AvrXa7, supporting the strong evolutionary pressure imposed by the pathogen's population on this gene. Nonetheless, we identified four accessions of *Oryza barthii* with a 15bp deletion potentially affecting the binding activity of AvrXa7 and Tal5, which originate from the Philippines and Mali, respectively. We next demonstrated that the *O. barthii* accessions provide resistance to strains of Xoo relying on Tal5 and AvrXa7 for virulence, due to the unresponsiveness of the SWEET14 promoter specifically. Finally, additional Xoo strains representative of the pathovar population diversity worldwide were also tested for virulence. Our preliminary data suggest that the new SWEET14 O. barthii alleles may be useful for

alternative broad and durable control of BLB applying impaired Effector-Triggered Susceptibility strategies.

Search for the genes encoding proteins with antifungal potential

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Despite the great progress in plant disease control strategies, the food supply in the world is still threatened by a multitude of pathogens and pests. Therefore much effort has been invested towards understanding innate resistance mechanisms in plants. One of the approaches involves the study of so-called hypersensitive reaction which leads to expression of pathogenesis-related (PR) proteins. When some of these proteins are over-expressed in transgenic plants, they are able to reduce the symptoms of plant diseases, depending on the nature of the hydrolytic protein, recipient plant species and pathogen involved.
The goal of this research is focused on testing of genes for "pathogenesis-related" (PR) proteins coming from phylogenetically distant plant species *Drosera rotundifolia* for enhancement of transgenic plant defence. Here we bring *in silico* characterization of isolated chitinase gene, preparation of vector construct for strong constitutive expression in plants and following the *Agrobacterium tumefasciens* transformation - generation of transgenic plants. Later when the transgenic plants are characterized on molecular level their plant extracts will be tested for altered ability to inhibit the growth of tested phytopathogenic fungi in conditions in vitro.
The results of research contribute to the search of desired genes with perspective application in plant transgenesis.

Breeding for improved resistance to *Stagonospora nodorum* blotch in wheat by elimination of sensitivity to necrotrophic effectors.

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Leaf blotch caused by *Stagonospora nodorum* (teleomorph *Phaeosphaeria nodorum*) is a severe disease on wheat in Norway and other areas with a temperate and rainy climate. Insufficient resistance causes severe grain shriveling and reduced yield in years with favorable conditions for the disease, and is a main cause of fungicide application.
S. nodorum and other necrotrophic leaf blotch pathogens interact with their hosts in an inverse gene-for-gene manner based on necrotrophic effectors (NEs, also known as host-selective toxins) that are recognized by corresponding sensitivity loci in the host. These sensitivity loci are thought to be resistance (R) genes to biotrophs that are "hijacked" by the necrotrophic pathogen to trigger programmed cell death. To date, six NEs and corresponding host sensitivity loci have been described for the wheat - *S. nodorum* pathosystem. This has opened up new possibilities in resistance breeding by identification and elimination of host receptors.
This presentation will review the current progress in the identification of NE sensitivity loci in wheat and prospects for the use of molecular markers and other tools to enhance resistance breeding to this challenging plant pathogen.

***Agrobacterium tumefaciens* infection affects the reproduction of *Meloidogyne ethiopica* root-knot nematodes**

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Agrobacterium tumefaciens is a widespread soil bacterium with a saprophyticlifestyle. Strains carryingthe Ti plasmid are pathogenic andcause crown gall disease manifested as tumorson the plant crown or on the roots. Visually, the tumors on the host roots looksimilar to the galls of root-knot nematodes. The present study explored the influence of *A. tumefaciens* on reproduction of *M. ethiopica* on roots of tomato hybrid Arawak F1 (Syngenta). The reproduction of *M. ethiopica* was not affected when bacteria were co-inoculated with the nematodes and when bacteria where added one week after the nematodes. The nematode reproduction was significantly decreased when nematodes were inoculated on roots already colonized by *A. tumefaciens*. The reduction was twofold, significant ($p < 0.001$). The effect was similar with a non-pathogenic agrobacterial strain that does not cause crown gall. We hypothesise that the mechanism responsible for the antagonistic interaction might be systemic resistance response activated by the host recognition of bacteria. The aim of our further studies will be determination of the plant response in the plant-nematode-bacteria interaction. We will examine gene expression of certain genes involved in pathogen recognition and different hormonal pathways with a quantitative real-time PCR. The mechanism of plant response to bacterial infection and suppressing nematode reproduction may present the potential for developing nematode management strategy.

The durum wheat leaf rust resistance locus Lr14a maps in a chromosome region enriched in plant-pathogen recognition genes

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Leaf rust (*Puccinia triticina* Eriks. & Henn.) is a major disease affecting durum wheat production. The Lr14a-resistant gene present in the durum wheat cv. Creso and its derivative cv. Colosseo is one of the best characterized leaf-rust resistance sources deployed in durum wheat breeding. Lr14a has been mapped close to the SSR markers gwm146, gwm344 and wmc10 in the distal portion of the chromosome arm 7BL, a gene-dense region. The objectives of this study were: (1) to enrich the Lr14a region with single nucleotide polymorphisms (SNPs) and high-resolution melting (HRM)-based markers developed from conserved ortholog set (COS) genes; (2) to further investigate the gene content and colinearity of this region with the *Brachypodium* and rice genomes. Ten new COS-SNP and five HRM markers were mapped within an 8.0 cM interval spanning Lr14a. Eight COS-SNPs were mapped in an interval of 4.1 cM including Lr14a, between markers YP7B-1 and ubw35. This region corresponds to a well-defined syntenic interval in both *Brachypodium* chromosome 1 and rice chromosome 6, where several genes involved in plant-pathogen recognition and interaction are located. Among such genes, the most interesting ones are: Bradi1g29537 (cyclin-L1-1domain), Bradi1g29530 (zinc knuckle domain), Bradi1g29471 (serine carboxypeptidase homolog) and Bradi1g29450, an NBS-LRR disease resistance protein homolog of Yr10 and present in three copies. All of these genes are potentially involved in the disease-response mechanisms. Fine mapping activities and the functional characterization of those genes is underway to provide insights on the

mechanisms underlying the involvement of this chromosome regions in wheat defense mechanism.

Towards the fine mapping and definition of the gene-content of QSbm.ubo-2B, a QTL for resistance of durum wheat to Soil-Borne Cereal Mosaic Virus (SBCMV)

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Genetic analysis of Soil-Borne Cereal Mosaic Virus (SBCMV) resistance in durum wheat, a viral disease widely spread in Italy, France and Spain, was carried out with two recombinant inbred line (RIL) mapping populations from Meridiano (resistant) × Claudio and Simeto (susceptible) × Levante (resistant). The RILs were characterized for SBCMV response under severe infection conditions over three consecutive years (2007-2009) of field trials. In both populations, a major quantitative trait locus (QSbm.ubo-2BS) in the distal chromosome 2BS accounted for 60–90% of the phenotypic variation for symptom severity, 40–70% for virus concentration and 30-60% for grain yield. By means of meta-QTL analysis QSbm.ubo-2BS was mapped as a unique QTL within a 2 cM-wide interval (LOD-2) close to the DArT marker wPt-2106 and SSR markers wmc661-gwm210-barc35. Fine mapping of the QTL required considerable efforts due to the low polymorphism level. The sequence of the DArT marker wPt-2106, co-segregating with the Mendelized QTL in the M×C population, was used to identify and sequence a durum wheat BAC clone in order to identify candidate genes. The recently developed Illumina wheat iSelect 90K SNP array allowed us to map seven additional SNPs derived from wheat coding sequences that co-segregate with the QTL. The fine mapping and the investigation of the gene content at the target region is underway.

Identifying and classifying trait linked SNPs in non-reference species by walking coloured De Bruijn graphs

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Single Nucleotide Polymorphisms are invaluable markers for tracing the genetic basis of inheritable traits and the ability to create marker libraries quickly is vital for timely identification of target genes. Next-generation sequencing makes it possible to sample a genome rapidly, but polymorphism detection relies on having a reference genome to which reads can be aligned and variants detected. We present Bubbleparse, a method for detecting variants directly from next-generation reads without a reference sequence. Bubbleparse uses the de Bruijn graph implementation in the Cortex framework as a basis and allows the user to identify bubbles in these graphs that represent polymorphisms, quickly, easily and sensitively. The Bubbleparse algorithm is sensitive, can detect many polymorphisms quickly and performs well when compared with polymorphism detection methods based on alignment to a reference in Arabidopsis thaliana and found some SNPs not found by the canonical method. The heuristic can be used to maximise the number of true polymorphisms returned and with a proof-of-principle experiment we show that Bubbleparse is very effective on data from unsequenced wild relatives of potato and enabled us to identify disease resistance linked genes quickly and easily. Bubbleparse is a fast and effective tool for detection of polymorphisms in unsequenced

genomes and is an excellent addition to the genomics toolbox, it can speed up variant detection and allow for new analyses in organisms that do not as yet have substantial genomic resources.

Targeting resistance effectors of rust and powdery mildew resistance in oat by association analysis

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Biotic stresses such as rust and powdery mildew constitute major constraints for cereal crops. The purpose of this work was to identify elite alleles for rust and powdery mildew resistance in oat by association mapping to further tackle the effectors responsible of the resistance phenotype. To this aim, 176 oat accessions including white and red oat cultivars and landraces were evaluated for disease resistance and further genotyped with 31 simple sequence repeat (SSR) and 15,000 Diversity Arrays Technology (DArT) markers to reveal association with disease resistance traits. 1872 polymorphic markers combining DArT and SSR markers were considered for association analysis. Population structure was inferred using two different methods; Principal Component Analysis and a Bayesian clustering. In adittion, five different general and mixed linear models accounting for population structure and/or kinship corrections and two different statistical tests were carried out to reduce false positive. This analysis identified 7 marker alleles strongly associated with rust resistance. For powdery mildew resistance, only 1 marker at the limit of significance was found, probably due to the low phenotypic variation detected in the collection for powdery mildew resistance. Since the sequence of the DArT markers are known and long enough for blast analysis, we are currently carrying out blast of associated markers sequences for determining putative effectors associated with resistance to these biotrophic fungi in oat.

Stacking two late blight resistance genes Rpi-blb1 and Rpi-blb3 into potato cultivars by somatic hybridization between commercial varieties and the incongruent species *Solanum bulbocastanum*

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The wild species of *Solanum* growing in potato's center of origin represent a rich reservoir of resistance genes, but are sexually incompatible with cultivated *Solanum tuberosum*. In the last years many resistance genes to *Phytophthora infestans* (Pi) were identified, sequenced and cloned. *S. bulbocastanum* (S. blb) accessions proved to be the source of four such genes: Rpi-blb1 (RB), Rpi-blb2, Rpi-blb3 and Rpi-bt1. The goal of our research was to characterize a resistant *S. bulbocastanum* accession, to clone it *in vitro* and to produce by electrofusion a large number of somatic hybrids (SHs) with five commercial potato varieties. From two combination, with cultivars 'Delikat' and 'Rasant', fertile and resistant somatic hybrids were selected to produce backcross generations: BC1 and BC2. The analysis by using gene-specific primers demonstrated the presence of resistance genes Rpi-blb1 and Rpi-blb3 in the wild species

accession and their transfer and introgression into SHs and subsequent BC generations. BC progenies carrying both genes and resistant to foliage blight could be selected. The resistance of the SHs and BC progeny with the two genes: Rpi-blb1 and Rpi-blb3 to foliage late blight was revealed by the detached leaf assay (DLA) and field trials. The presence of both genes strongly correlates with resistance in DLA assays (r = 0.9). Tuber yield in field trials was similar to that of the cultivars. This is the first report of somatic hybridization being used to successfully stack two resistance genes into commercial cultivars of potato.

Peptide aptamer-mediated resistance to barley powdery mildew.

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Biotrophic fungi, such as *Blumeria graminis f. sp. hordei* (Bgh), develop structures (haustoria) in the living plant cells. Around each haustorium, the host cell generates an extrahaustorial membrane (EHM). In order to control the host plant, fungi secrete effector proteins that are believed to inhibit host defense mechanisms. Approximately 500 barley powdery mildew effector candidate proteins have been identified, showing no homology to any known proteins outside the Bgh genome (Pedersen et al., 2012). The only common feature of those putative effectors is YxC-motif in the N-terminal of the mature protein (Godfrey et al., 2010). This feature makes it a very interesting target in engineering disease resistance. Peptide aptamers are an excellent tool for this purpose, because of their small size, high recognition specificity and high binding affinity to their target (Colas, 2008).
In this study, we identified peptide aptamers that target the YxC motif of CSEPs in order to obtain powdery mildew resistance. YxC-binding peptide aptamers were found in yeast two-hybrid screens of peptide aptamer libraries (Stadler et al., 2011). Analysis of peptide aptamer-YxC motif interactions showed it to be YxC-motif specific, which suggests that the identified peptide aptamers should be able to target the majority of CSEPs. A few YxC-binding peptide aptamers were transiently overexpressed in barley epidermal cells, which subsequently showed to be less susceptible to Bgh. Future work will include bimolecular complementation assays of selected peptide aptamers together with YxC CSEPs to confirm that interaction in planta.
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