



VISCEA

Vienna International Science
Conferences and Events Association

International Conference

**Plant Biotic Stresses &
Resistance Mechanisms IV**



Programme & Abstracts

February 19-20, 2020



International Conference

**Plant Biotic Stresses & Resistance
Mechanisms IV**

Programme and Abstracts

Vienna, Austria

February 19 – 20, 2020

Organizing Committee

Local Organizing Committee	International Organizing Committee
Alisher Touraev (Local Organizer, Austria) Frank Takken (Conference Co-Chair, The Netherlands)	Niko Geldner (Switzerland) Sebastian Schornack (United Kingdom) Takaki Maekawa (Germany) Bostjan Kobe (Australia) Thomas Kroj (France) Alga Zuccaro (Germany) Steven Spoel (United Kingdom) Corina Vlot (Germany) Gitta Coaker (USA) Renier van der Hoorn (United Kingdom) Guido van den Ackerveken (The Netherlands) Harrold van den Burg (The Netherlands) Brande Wulff (United Kingdom) Vivianne Vleeshouwers (The Netherlands)

Welcome to the 4th International Conference on “Plant Biotic Stresses & Resistance Mechanisms”!

Despite that the modern crops are mainly intensive, high yield with good resistance to biotic and abiotic stresses, in some regions up to 30% yield are lost every year because of diseases or other stresses. Biotic Stress occurs as a result of damage done to plants by other living organisms, such as bacteria, viruses, fungi, parasites, beneficial and harmful insects, weeds, and cultivated or native plants. Therefore, understanding the mechanisms of resistance to plant biotic stress and plant diseases is one of the hottest areas of modern plant science.

The **4th International Conference “Plant Biotic Stresses & Resistance Mechanisms”** to be held on **February 19-20, 2020**, in Vienna, Austria will discuss the most recent advances in understanding and combating plant biotic stress and resistance mechanisms and to define new frontiers in this field.

This two-days event will provide leading academy and industry scientists a platform to communicate recent advances in **“Plant Biotic Stresses & Resistance Mechanisms”**, and an opportunity to establish multilateral collaboration.

The **4th International Conference on “Plant Biotic Stresses & Resistance Mechanisms”** will cover the following research topics:

- ***NLR Structure & Signaling Mechanisms***
- ***Immune Signaling***
- ***Endophyte Induced Plant Immunity***
- ***Immune Signaling and Plant Hormones***
- ***Role of Effectors in Host Manipulation***
- ***Translation Research in Plant Immunity***
- ***Omics in Plant Immunity***

Approximately 150 participants are expected to attend this exciting scientific forum including almost 30 lectures delivered by worldwide known invited speakers and young, talented speakers selected from submitted abstracts. The program combines plenary lectures, poster sessions, a unique Conference Dinner Party and sightseeing tours of Vienna.

Prof. Alisher Touraev (VISCEA, Austria, Local Organizer)

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4th International Conference on “Plant Biotic Stresses & Resistance Mechanisms”
(February 19 - 20)

February 19 (Wednesday)

08.00 - 17.00	Registration
	Opening
09.00 - 09.10	Welcome address by Alisher Touraev (Local Organizer, Austria) Welcome address by Frank Takken (Conference Co-Chair, The Netherlands)
	Keynote Lecture:
09.10 - 09.50 (+5)	Niko Geldner (Switzerland): Root Damage and Immune Responses at Cellular Resolution
	Keynote Lecture:
09.55 - 10.35 (+5)	Sebastian Schornack (United Kingdom): Non-Vascular Plants as Models for Plant-Microbe Interactions
10.40 - 11.00	Coffee break
11.00 - 12.30:	Session I: NLR Structure & Signaling Mechanisms
<i>Chairs</i>	<i>Bostjan Kobe (Australia) & Takaki Maekawa (Germany)</i>
11.00 - 11.25 (+5)	Bostjan Kobe (Australia): Plant NLR TIR Domains Possess NAD ⁺ -Cleavage Activity
11.30 - 11.55 (+5)	Takaki Maekawa (Germany): Plant Mixed Lineage Kinase Domain-like Proteins Limit Biotrophic Pathogen Growth
12.00 - 12.25 (+5)	Kee Hoon Sohn (Republic of Korea): RIN4 Natural Variants Carry Distinct Properties for Defense Activation
12.30 - 14.00	Lunch + Poster Session (all numbers), Conference Photo
14.00 - 15.20	Session II: Immune Signaling
<i>Chairs</i>	<i>Thomas Kroj (France) & Guido Sessa (Italy)</i>
14.00 - 14.25 (+5)	Thomas Kroj (France): Pathogen Effector Recognition by Plant NLR Immune Receptors and Decoy Domains
14.30 - 14.50 (+5)	Guido Sessa (Italy): The Tomato Receptor-like Cytoplasmic Kinase BSK830 Associates with Immune Receptors and Plays a Role in PTI
14.55 - 15.10 (+5)	Kathrin Thor (United Kingdom): Identification of a Calcium-Permeable Channel which Mediates Stomatal Immunity
15.10 - 15.25 (+5)	Jennifer Sales (Germany): LEGUME LECTIN-LIKE PROTEINS at the Interface of Systemic Immunity and Abiotic Stress
15.30 - 16.00	Coffee break
16.00 - 17.35	Session III: Endophyte Induced Plant Immunity
<i>Chairs</i>	<i>Alga Zuccaro (Germany) & Frank Takken (The Netherlands)</i>
16.00 - 16.25 (+5)	Alga Zuccaro (Germany): Beneficial Root Endophyte Interactions in Barley and Arabidopsis: Immunity, Metabolism and Cell Death

16.30 - 16.55 (+5)	Frank Takken (The Netherlands): Molecular Aspects of Endophyte-Mediated Resistance induced by <i>Fusarium oxysporum</i>
17.00 - 17.10 (+5)	Andrea Romero Perez (Belgium): <i>Pseudomonas Syringae</i> Infection in <i>Arabidopsis thaliana</i> : The Role of F-Box Nictaba
17.15 - 17.25 (+5)	David Percival (Canada): Comparing the Response of Pr Genes in wild Blueberry Phenotypes challenged with <i>Botrytis Cinerea</i>
17.30 - 19.00	<i>Welcome Reception + Poster Session (all numbers)</i>
19.00 - 22.00	Conference Dinner Party Traditional Austrian food and wine, located in one of Vienna's famous 'Heurigen' Cost: 50,- EUR

February 20 (Thursday)

08.00 - 17.00	<i>Registration</i>
<u>09.00 - 10.30</u>	<u>Session IV: Immune Signaling and Plant Hormones</u>
<i>Chairs</i>	<i>Steven Spoel (UK) & Corina Vlot (Germany)</i>
09.00 - 09.25 (+5)	Steven Spoel (UK): Ubiquitin Signaling in Plant Immunity
09.30 - 09.55 (+5)	Corina Vlot (Germany): Volatile Terpenes in SA-Associated Immunity
10.00 - 10.10 (+5)	Anna Kulma (Poland): The non-mevalonate Pathway of Terpenoid Synthesis Activation in Flax upon <i>Fusarium oxysporum</i> Infection results in increased ABA synthesis
10.15 - 10.25 (+5)	Alan Wanke (Germany): Recognition of beta-glucans and Consequent Immune Responses vary among Plant Species
10.30 - 11.00	<i>Coffee break</i>
<u>11.00 - 12.30</u>	<u>Session V: Role of Effectors in Host Manipulation</u>
<i>Chairs</i>	<i>Gitta Coaker (USA) & Renier van der Hoorn (UK)</i>
11.00 - 11.25 (+5)	Gitta Coaker (USA): Host Manipulation by Phloem-Limited Bacteria
11.30 - 11.55 (+5)	Renier van der Hoorn (UK): The Hydrolytic Battlefield at the Plant-Pathogen Interface
12.00 - 12.10 (+5)	Adam Bentham (UK): Adaptive Evolution at a Pathogen Effector-host Target Binding Interface is Associated with Host Specificity
12.15 - 12.25 (+5)	Tatiana Marti Ferrando (The Netherlands): The Receptor of the Apoplastic Effector SCR74 of <i>Phytophthora infestans</i> is mapped to a Receptor-like Kinase Cluster in <i>Solanum microdontum</i>
12.50 - 14.00	<i>Lunch + Poster Session (all numbers)</i>
<u>14.00 - 15.30</u>	<u>Session VI: Translation Research in Plant Immunity</u>
<i>Chairs</i>	<i>Guido van den Ackerveken (The Netherlands) & Harrold van den Burg (The Netherlands)</i>
14.00 - 14.25 (+5)	Guido van den Ackerveken (The Netherlands): Susceptibility Genes as Targets for Disease Resistance Breeding

14.30 - 14.50 (+5)	Harrold van den Burg (The Netherlands): Protein Modifier SUMO Recruits E2 Conjugating Enzyme (SCE1) to the Replication Initiator Protein to allow Replication of the Geminivirus TYLCV
14.55 - 15.10 (+5)	Juan de-La-Concepcion (UK): Protein Engineering Expands the Effector Recognition Profile of a Plant NLR Immune Receptor
15.15 - 15.30 (+5)	Katarina Šoln (Slovenia): Allelopathic Plant-Plant Interactions: The Additional Mechanism of the Dominance of Invasive Japanese and Bohemian Knotweed
15.35 - 16.00	<i>Coffee Break</i>
<u>16.00 - 17.30</u>	<u>Session VII: Omics in Plant Immunity</u>
<i>Chairs</i>	<i>Brande Wulff (UK), Vivianne Vleeshouwers (The Netherlands)</i>
16.00 - 16.25 (+5)	Brande Wulff (UK): Understanding and Exploiting Immune Receptors in Wheat and their wild Relatives
16.30 - 16.55 (+5)	Vivianne Vleeshouwers (The Netherlands): Effector-Driven Breeding for Disease Resistance in Potato
17.00 - 17.10 (+5)	Agnieszka Żmieńko (Poland): Copy Number Variation of Genes involved in Biotic Stress Interactions in a Model plant Arabidopsis thaliana
17.15 - 17.30	<i>Closing Ceremony</i>



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Abstracts of Oral Presentations



Root damage and immune responses at cellular resolution

Niko Geldner

University of Lausanne, Switzerland

Correspondence to: niko.geldner@unil.ch

Microbe-associated molecular pattern (MAMP) recognition is crucial to the plant's immune system, but how this sophisticated perception system can be usefully deployed in roots, continuously exposed to bacteria, remains unresolved. We have analyzed MAMP receptor expression and responses at cellular resolution in Arabidopsis and found that differentiated outer layers, exposed to bacteria, show low receptor levels and lack MAMP responsiveness. However, these cells can be locally "gated" to become responsive, by either neighbor cell damage or emerging lateral roots. Laser-induced localized damage also leads to immune responses to an otherwise non-immunogenic, beneficial bacterium and enhances responses to a root pathogenic bacterium. Moreover, we find that single cell damage in roots leads to regional ROS and calcium waves, ethylene responses, but no detectable jasmonate responses. Treatment with DAMPs alone do not re-iterate laser-induced damage and, surprisingly, the very local upregulation of MAMP responses by damage is independent of ethylene signalling. Our findings demonstrate that spatially restricted receptor expression is crucial for an appropriate MAMP response in roots and helps to conceptualize how MAMP perception can be used despite a continuous presence of microbial patterns in the soil.

Non-vascular plants as models for plant-microbe interactions

Sebastian Schornack

Correspondence to: sebastian.schornack@slcu.cam.ac.uk

Our food crops are vascular flowering plants and hence most of our understanding of processes underlying plant-microbe interactions originates from flowering plant model systems such as Arabidopsis. However, the green lineage consists of many divergent plants which early on during land plant evolution followed their own trajectories. Recently, comparative studies of non-vascular bryophytes and flowering plants have helped gaining a better understanding of conserved principles in plant biology. The reduced genome complexity of non-vascular liverworts such as the model plant *Marchantia polymorpha* supports fast gene to phenotype linking and therefore enables new discoveries such as common and divergent plant-microbe processes from the organ- down to the cell level. I will introduce the *Marchantia* and related bryophytes as systems to study the interaction with beneficial and detrimental filamentous microbes and highlight recent findings such as natural variation in oomycete liverwort interactions, the transcriptional and proteomic response to infection by an oomycete and evolutionarily conserved features indicative of an ancestral pathogen deterrence strategy centered on phenylpropanoid-mediated biochemical defenses.

The ZAR1 resistosome: a large protein complex mediating plant immunity

Jijie Chai

University of Cologne, Cologne, DE; Max Planck Institute for Plant Breeding Research, Cologne, DE; Tsinghua University, Beijing, China.

Correspondence to: chai@mpipz.mpg.de

Nucleotide-binding domain leucine-rich repeat (NLR) proteins function as immune receptors in both animals and plants. Specific detection of pathogen effectors by NLR receptors play a crucial role in plant immunity. ZAR1 is a coiled-coil (CC)-NLR in Arabidopsis and mediates resistance to the *Xanthomonas campestris* bacteria carrying the uridylylase effector AvrAC by pre-complexing with the RKS1 pseudokinase. AvrAc-uridylylated PBL2 (PBL2UMP) interacts with RKS1, inducing ZAR1-mediated immunity. Our structural, biochemical and functional studies revealed the mechanisms of ZAR1 autoinhibition and ZAR1-RKS1 recognition of PBL2UMP. PBL2UMP binding allosterically induces ADP release from ZAR1 and thus primes its activation. More importantly, in the presence of ATP or dATP, the primed ZAR1 complex oligomerizes into a wheel-like pentamer as revealed by cryo-EM structure. Assembly of the pentameric ZAR1 complex, which we term the 'ZAR1 resistosome', results from structural reorganization and fold switching of ZAR1 during activation. These changes free an N-terminal helix of ZAR1 that is completely buried prior to activation. The five N-terminal helices form a funnel-shaped structure in the ZAR1 resistosome. Functional studies support an indispensable role of the funnel-shaped structure in plasma-membrane association, cell death triggering and disease resistance. Our data suggest that the ZAR1 resistosome may function as a channel or pore to execute cell death and immune response.

Plant mixed lineage kinase domain-like proteins limit biotrophic pathogen growth

Takaki Maekawa

Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany

Correspondence to: maekawa@mpipz.mpg.de

Mixed lineage kinase domain-like (MLKL) protein mediates necroptotic cell death in vertebrates. We report here the discovery of a conserved protein family across seed plants that is structurally homologous to vertebrate MLKL. The *Arabidopsis thaliana* genome encodes three MLKLs with overlapping functions in limiting growth of obligate biotrophic fungal and oomycete pathogens. Although displaying a cell death activity mediated by N-terminal helical bundles, termed HeLo domain, AtMLKL-dependent immunity can be separated from host cell death. Cryo-electron microscopy structures of AtMLKLs reveal a tetrameric configuration, in which the pseudokinase domain and brace region bury the HeLo-domains, indicative of an auto-repressed complex. We also show the association of two AtMLKLs with microtubules. These findings, coupled with resistance-enhancing activity and altered microtubule association of a phosphomimetic mutation in the pseudokinase domain of AtMLKL1, point to a cell death-independent immunity mechanism.

RIN4 natural variants carry distinct properties for defense activation

Kee Hoon Sohn

Postech Biotech Center, 77 Cheongam-Ro, Postech, Pohang, Republic of Korea

Correspondence to: khsohn@postech.ac.kr

Plant innate immunity relies on two layers of pathogen detection. Cell surface-localized pattern recognition receptors detect pathogen-associated molecular patterns (PAMPs) of invading microorganisms and activate PAMP-triggered immunity (PTI). Successful pathogens must circumvent PTI to colonize plants, and many bacterial pathogens use type III secretion (T3S) to deliver effectors that suppress PTI into plant cells. Effectors can be detected directly or indirectly by plant disease resistance (R) proteins, which then activate effector-triggered immunity (ETI) generally together with a hypersensitive response (HR) of the infected tissue. RPM1-interacting protein 4 (RIN4) is an important regulator of plant basal immunity and targeted by multiple bacterial effectors. It is well-known that effector-directed biochemical modifications (e.g. proteolytic cleavage, ADP-ribosylation) of RIN4 activate corresponding NLR (nucleotide-binding and leucine rich repeat resistance protein) immune receptors in plants. However, how naturally occurring RIN4 variants function is unclear. We have analyzed multiple RIN4 natural variants originated from diverse plant species for their ability to activate or suppress the previously known RIN4-associated NLRs. Several previously unknown properties of RIN4 in defense activation will be presented.

Plant NLR TIR domains possess NAD⁺-cleavage activity

Bostjan Kobe¹, Hayden Burdett¹, Xiaoxiao Zhang^{1,2,3}, Jian Chen², Maxwell X. Rank¹, Shane Horsefield¹, Mohammad K. Manik¹, Yun Shi⁴, Thomas Ve⁴, Peter N. Dodds²

¹School of Chemistry and Molecular Biosciences, University of Queensland, Brisbane, QLD 4072, Australia

²Agriculture and Food, CSIRO, Canberra, ACT 2601, Australia

³Plant Sciences Division, Research School of Biology, The Australian National University, Canberra, ACT 2601, Australia

⁴Institute for Glycomics, Griffith University, Southport, QLD 4222, Australia

Correspondence to: b.kobe@uq.edu.au

A large group of plant NLRs contain TIR (Toll/interleukin-1 receptor, resistance) domains. The mechanism of signalling has remained unclear. TIR domains are also present in proteins involved in innate immunity and cell-death signaling pathways in animals. We have therefore been able to learn about the molecular mechanisms of signaling by TIR domains by complementary studies of both animal and plant systems. We reconstituted large assemblies of the TLR (Toll-like receptor) adaptor TIR domains and determined the structures of filamentous assemblies by cryo-electron microscopy. We further determined the crystal structure of the TIR domain from the protein SARM1, involved in axon degeneration, a cell-death process operating in neurons that involves cleavage of the dinucleotide NAD⁺. These studies have informed on the function of plant TIR domains. We have evidence that plant TIR domains can also self-associate through more than one interface. Structural similarities between SARM1 and plant TIR domains led us to demonstrate that plant TIR domains can cleave NAD⁺, and this activity likely plays a role in their HR function.

Pathogen effector recognition by plant NLR immune receptors and decoy domains

Thomas Kroj

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

Correspondence to: thomas.kroj@inra.fr

NLRs are an important class of plant immune receptors that mediate specific recognition of pathogen effectors inside host cells by the formation of protein complexes. They are characterized by a multi-domain architecture composed of a variable N-terminal domain, a central nucleotide-binding (NB-ARC) domain and C-terminal leucine-rich repeats (LRR). Based on our work on the detection of effectors from the pathogenic fungus *Magnaporthe oryzae* by the rice NLR RGA5, we developed the hypothesis that some NLRs recognize effectors through non-canonical integrated domains (IDs) that act as mimics of effector targets. By genomic analysis, we showed that NLRs carrying integrated decoy domains are frequent and widespread in plant genomes. This provides new ways to identify effector targets and immunity-related genes in plant genomes. By precise structure-function analysis, we started to decipher the molecular details of effector recognition by RGA5, and I will show how we use this knowledge for the engineering of plant immune receptors with novel recognition specificities.

The tomato receptor-like cytoplasmic kinase BSK830 associates with immune receptors and plays a role in PTI

Guido Sessa, Guy Sobol¹, Bharat Bhusan Majhi¹, Ning Zhang², Holly M. Roberts², Gregory B. Martin², Guido Sessa¹

¹ Tel-Aviv University, 69978 Tel-Aviv, Israel.

² Boyce Thompson Institute for Plant Research, Ithaca, NY 14853, USA.

Correspondence to: guidos@tauex.tau.ac.il

Brassinosteroid signaling kinases (BSK) are receptor-like cytoplasmic kinases with established functions in growth and development. We investigated the role of tomato BSK830 in pattern-triggered immunity (PTI). BSK830 is anchored to the plasma membrane through myristoylation and palmitoylation, and interacted in yeast with the FLS2, FLS3, and Bti9 pattern recognition receptors. These interactions were validated in planta and treatment with the PAMP flgII-28 reduced the BSK830-FLS3 interaction. Consistent with a role in PTI, CRISPR/Cas9 *bsk830* mutants and BSK830-YFP overexpression lines displayed reduced production of ROS, but unaltered MAPK activation, upon flgII-28 treatment. In addition, mutant and overexpression lines were less protected than wild-type plants against infection of *Pseudomonas syringae* pv. tomato bacteria by pretreatment with non-pathogenic *Pseudomonas fluorescens* bacteria. *bsk830* mutants also displayed enhanced susceptibility to the fungal pathogen *Botrytis cinerea*. To start investigating if BSK830 is targeted by bacterial type III effectors, we analyzed the interaction of BSK830 with 35 *Xanthomonas euvesicatoria* effectors. This analysis identified 7 effectors that interacted with BSK830. Together, our results support the hypothesis that BSK830 plays a role in PTI and is a target of bacterial effectors.

Identification of a calcium-permeable channel which mediates stomatal immunity

Kathrin Thor¹, Shushu Jiang¹, Erwan Michard², Paul Derbyshire¹, Frank Menke¹, Dan MacLean¹, José Feijo², Cyril Zipfel^{1,3}

¹The Sainsbury Laboratory, Norwich Research Park, Norwich NR4 7UH, UK

²University of Maryland Department of Cell Biology and Molecular Genetics, College Park, MD 20742-5815, USA

³Institute of Plant and Microbial Biology and Zurich-Basel Plant Science Center, University of Zurich, 8008 Zurich, Switzerland

Correspondence to: kathrin.thor@tsl.ac.uk

Plant defence against pathogens is a multi-layered process, which involves recognition of pathogen- or damage-associated molecular patterns (PAMPs or DAMPs) by their corresponding receptors, which is followed by a rapid increase in the cytosolic calcium concentration [Ca²⁺]_{cyt}. This calcium signal is decoded into downstream responses, an important one being the closure of stomata. We have identified a calcium-permeable channel, which is rapidly phosphorylated upon PAMP-treatment and required for stomatal closure. Phosphorylation thereby increases the channel activity and is important for the closing reaction. Moreover, corresponding mutants do still close their stomata upon treatment with the abiotic stress-related hormone ABA, pointing to a specificity in calcium-influx mechanisms upon different kinds of stresses.

LEGUME LECTIN-LIKE PROTEINS at the interface of systemic immunity and abiotic stress

Sales Jennifer¹, Vlot Corina A.C.¹

¹Institute of Biochemical Plant Pathology, Helmholtz Center Muenchen

Correspondence to: jennifer.sales@helmholtz-muenchen.de

Systemic Acquired Resistance (SAR) is a broad spectrum induced defence response against biotrophic pathogens following a local infection. LEGUME LECTIN-LIKE PROTEIN 1 (LLP1) is required in distal tissues to recognise both phloem mobile and air-borne SAR signals. LLP1 shares a high sequence homology with two other proteins, LLP2 and LLP3, which have not yet been studied with respect to systemic defence. Here, we present RNAi lines in which the transcript accumulation of LLP1, LLP2, and LLP3 is reduced. Although local defence responses remain unchanged, the transgenic lines show increased susceptibility to *Pseudomonas syringae* in their systemic tissue, suggesting that multiple LLPs act in concert during SAR. The lack of LLPs also confers susceptibility to a necrotrophic pathogen, and compromises jasmonate-mediated salt tolerance independently of their previously known roles in SAR signalling pathways. Transcript accumulation of the SA marker gene PATHOGENESIS-RELATED1 was induced by JA in the RNAi lines, while *llp1* mutants responded to a local JA treatment with elevated systemic resistance to *P. syringae*. This opens the recognised dual but distinct systems of salicylic acid and piperolic acid pathways for reinterpretation, and supports a role for JA in systemic immune signalling.

Beneficial Root Endophyte Interactions in Barley and Arabidopsis: Immunity, Metabolism and Cell Death

Alga Zuccaro

Correspondence to: azuccaro@uni-koeln.de

Cell death is intricately connected with life in multicellular organisms. The balance between cell death, proliferation and differentiation shapes organ development and is critical for the maintenance of tissue homeostasis throughout life. Cell death also plays a central role in host-microbe interactions and pathogen defense. Colonization by the beneficial root endophyte *Serendipita indica* follows a biphasic strategy. After a biotrophic phase the fungus switches to a cell death associated phase restricted to the epidermal and first cortex layers which is needed for fungal accommodation in the roots and the establishment of symbiosis in barley and *Arabidopsis*. Using a combination of biochemical and cytological analyses, transcriptomics and proteomics, we have identified and functionally characterized several fungal derived apoplastic proteins involved in the subversion of the plant immune system and metabolism and in the manipulation of host cell death. An overview about these mechanisms will be given during the talk.

Molecular aspects of endophyte-mediated resistance induced by *Fusarium oxysporum*

Frank Takken, ME Constantin, FJ de Lamo, M de Sain, L Fokkens, PM Houterman, Ji-Ming L, M Rep
University of Amsterdam, Molecular Plant Pathology, Amsterdam, The Netherlands

Correspondence to: f.l.w.takken@uva.nl

With a host range of over 100 crop species, *Fusarium oxysporum* (Fo) is one of the most devastating fungal plant pathogens. It is therefore remarkable that the same fungus is used as a biocontrol agent to enhance plant tolerance to (a)biotic stresses. Co-inoculation of tomato roots with pathogenic and biocontrol Fo strains results in vasculature colonization of the host by the pathogen, but disease symptoms are strongly reduced. We are interested in how this protection is conferred, and what characteristics distinguish a pathogenic- from a biocontrol strain. Fo harbors dispensable chromosomes that in pathogenic strains are enriched for effector genes correlated with host range. Transfer of a pathogenicity chromosome to a biocontrol strain can convert it into a pathogen and vice versa. To determine the genetic requirements distinguishing them we determined the "effectorome" of 80 pathogenic and non-pathogenic Fo isolates to correlate key effectors with the ability to colonize tomato plants and/or cause disease. In addition, the xylem proteomes of (co)inoculated plants were determined to identify proteins involved in the interaction. The results of this ongoing analysis will be presented, along with a few examples of plant proteins and Fo effectors of which we have obtained some insight into their function in the tri-partite interaction.

Pseudomonas syringae infection in Arabidopsis thaliana: The role of F-Box Nictaba

Andrea ROMERO PÉREZ¹, Kris AUDENAERT², Maarten AMEYE², Els JM VAN DAMME¹

¹ Dept. Biotechnology, Ghent University, Belgium

² Dept. Plants and Crops, Ghent University, Belgium

Correspondence to: Andrea.RomeroPerez@UGent.be

F-box Nictaba (At2g02360) acts as a stress inducible lectin that can specifically recognize Lewis A motifs. Transgenic lines with enhanced F-box Nictaba expression were shown to be less susceptible to *Pseudomonas syringae* pv. *Tomato DC3000* infection. Our objective is to investigate the importance of F-box Nictaba and Lewis A epitopes for bacterial infection. We optimized a flooding assay to investigate the bacterial infection of two-week old *Arabidopsis* seedlings from different genotypes, including overexpression lines and knock out lines, generated using the CRISPR/Cas9 technology. Disease symptoms, qPCR analysis of transcript levels for F-box Nictaba and percentage of lesion area were studied for *Arabidopsis* leaves infected with wild type *Pseudomonas* strain and the mutant strain *fliC*. Symptoms at plant level were also investigated using the Pathoviewer, a plant phenotyping system that allows high-resolution multispectral imaging in a highly controllable environment. Finally, in order to monitor the infection process, a fluorescent strain of *Pst* was used to follow the entry of the bacteria in *Arabidopsis* plants using confocal microscopy.

Comparing the Response of Pr Genes in wild Blueberry Phenotypes challenged with Botrytis Cinerea

David Percival

Correspondence to: David.Percival@DAL.ca

Botrytis blight damage is variable in wild blueberry fields due to varying levels of genotypic resistance with more damage observed with *Vaccinium myrtilloides* than with *V. angustifolium* phenotypes. A field study was conducted to examine the molecular response and changes in resistance related genes during *Botrytis cinerea* infection. Flowers from six wild blueberry phenotypes were inoculated with a *B. cinerea* suspension. Tissues were harvested at 12 and 24 hrs after inoculation and pathogenesis related (PR) genes 3 and 4 were evaluated through real-time quantitative polymerase chain reaction. The expression levels of the PR-3 and PR-4 genes were generally induced and up-regulated in the flower tissues. Compared with the control (0 hr), the expression of PR3, was up-regulated in all the phenotypes at 24 hrs except *V. a. f. nigrum* where there was up regulation at 12 hrs which was higher than the up regulation at 24 hrs. Compared with the control (0 hr), the expression of PR4, was up-regulated in all the phenotypes at 12 and 24 hrs except *Vm* (tall) where there was down-regulation at both 12 and 24 hrs. PR4 was highly up regulated in *Va* (brown), *V. a. f. nigrum* and *V. m.* (medium) at 12 hrs but the expression reduced at 24 hrs. In *V. a.* (green) and *V. m.* (short) PR4 was highly expressed at 24 hrs than at 12 hrs. It was hypothesized that proteins such as chitinase (PR3 & 4) might play a role in the early stages of pathogenesis that notifies plants about the attack from a pathogen.

Ubiquitin signalling in plant immunity

Steven H. Spoel

Institute of Molecular Plant Sciences, School of Biological Sciences, University of Edinburgh, United Kingdom

Correspondence to: steven.spoel@ed.ac.uk

Gene expression plays pivotal roles in the development of eukaryotic cells and their response to the environment. Failure to precisely program cellular gene expression often has pathological or deleterious consequences. In plants, hormone-responsive transcriptional programs are controlled by nuclear E3 ubiquitin ligases that often function as both hormone receptors and as transcription cofactors. Indeed, E3 ligase-mediated ubiquitination events at or near the chromatin have been reported to regulate the stability of transcription activators and repressors, providing an ON/OFF switch for the control of gene expression. Our latest findings, however, challenge the simplicity of this model and instead demonstrate that dynamic ubiquitination by various ubiquitin-modifying enzymes determines transcription regulator activity. Upon subsequent arrival at the proteasome, transcription regulators undergo further ubiquitin chain remodelling, which determines their final fate. We will discuss how immune-induced cells dynamically utilise diverse ubiquitin chain linkages to fine-tune immune responses.

Volatile Terpenes in SA-Associated Immunity

A. Corina Vlot¹, Marion Wenig¹, Andrea Ghirardo², Jennifer Sales¹, Alessandro Brambilla¹, Yuanyuan Chen¹, Joerg-Peter Schitzler²

¹Helmholtz Zentrum Muenchen, Institute of Biochemical Plant Pathology, 85764 Neuherberg, Germany;

²Helmholtz Zentrum Muenchen, Institute of Biochemical Plant Pathology, Research Unit Environmental Simulation, 85764 Neuherberg, Germany

Correspondence to: corina.vlot@helmholtz-muenchen.de

Monoterpenes are volatile organic compounds that we recently associated with the salicylic acid (SA)-associated inducible resistance response systemic acquired resistance (SAR). SAR is induced in systemic tissues of plants undergoing a local SA-inducing infection. In addition, volatile emissions from infected tissues are recognized as defense cues by neighboring plants. In response to these plant-to-plant cues, receiver plants mount a SAR-like resistance response. Plant-to-plant cues contain monoterpenes, which are essential for both intra-plant SAR and plant-to-plant propagation of SAR-like resistance. In *Arabidopsis thaliana* monoterpene emissions appear to be induced downstream of other SAR-associated signals, including pipecolic acid and glycerol-3-phosphate. Monoterpene-induced resistance is further dependent on LEGUME LECTIN-LIKE PROTEIN1 (LLP1), which promotes SAR in parallel with SA. LLP1 drives a positive feedback loop in receivers of plant-to-plant cues, which results in the emission of further volatile cues. Ongoing research focuses on the regulation of monoterpene emissions during infection and after perception of plant-to-plant cues in both model and crop plants. Also, because LLP1 affects jasmonic acid (JA)-associated biotic and abiotic stress responses, we are currently testing if monoterpene-dependent inter-plant innate immune signaling is subject to SA-JA cross talk. Repercussions on the influence of SA, monoterpenes, LLP1, and JA on SAR will be discussed.

The non-mevalonate pathway of terpenoid synthesis activation in flax upon *Fusarium oxysporum* infection results in increased ABA synthesis

Aleksandra Boba¹, Kamil Kostyn², **Anna Kulma**¹

¹Faculty of Biotechnology, University of Wrocław, Przybyszewskiego 63/77, 51-148 Wrocław, Poland

²Department of Genetics, Plant Breeding and Seed Production, Faculty of Life Sciences and Technology, Wrocław University of Environmental and Plant Sciences, Plac Grunwaldzki 24A, 53-363 Wrocław, Poland

Correspondence to: aleksandra.boba@uwr.edu.pl

Plants have developed a number of defense strategies against the adverse effects of fungi such as *Fusarium oxysporum*. One such defense is the production of antioxidant secondary metabolites, which fall into two main groups: the phenylpropanoids and the terpenoids (sterols, carotenoids, tocopherols). Functions of phenylpropanoids and the activity of the genes involved in these compounds' synthesis during pathogen infection have been extensively studied. However, neither the functions of the isoprenoids, nor the expression of genes of the terpenoid biosynthesis after pathogen attack are clear. In order to broaden our knowledge of isoprenoid production and function in response to *Fusarium oxysporum* attack in flax, we investigated the changes in expressions of several genes involved in terpenoid synthesis triggered by pathogen attack. We discovered that in flax the non-mevalonate (MEP) pathway is strongly activated after pathogen infection. Changes of gene expression in the terpenoid pathway, especially of those connected with ABA synthesis are correlated with increased ABA level. As confirmed by our staining experiments, ABA may be connected with the early responses of flax to *F. oxysporum* infection.

Recognition of beta-glucans and consequent immune responses vary among plant species

Alan Wanke^{1,2}, Hanna Rovenich¹, Stephan Wawra¹, Florian Schwanke¹, Stefanie Velte¹, Stefan Becker³, Jan-Hendrik Hehemann³, Alga Zuccaro¹

¹ University of Cologne, Cluster of Excellence on Plant Sciences (CEPLAS), 50679 Cologne, Germany

² Max Planck Institute for Plant Breeding Research, 50829 Cologne, Germany

³ Center for Marine Environmental Sciences, University of Bremen, MARUM, 28359 Bremen, Germany

Correspondence to: alan.wanke@uni-koeln.de

Plants survey their environment for the presence of potentially harmful or beneficial microbes. During colonization, cell surface receptors perceive microbe-derived or modified-self ligands and initiate appropriate responses. The recognition of fungal chitin oligomers and the subsequent activation of plant immunity are well described. In contrast, the mechanisms underlying beta-glucan recognition and signaling activation remain largely unexplored. Here, we systematically tested immune responses towards different beta-1,3/1-6-linked glucan structures and show that responses vary between plant species. Our data shows that not the glycosidic decoration but rather the degree of polymerization plays a pivotal role in the recognition of beta-1,3-glucans. Moreover, in contrast to the recognition of short beta-1,3-glucan in *A. thaliana*, perception of long beta-1,3-glucan in *N. benthamiana* and rice is independent of CERK1, indicating that beta-glucan recognition may be mediated by multiple beta-glucan receptor systems. Additionally, we present data on the fungal exopolysaccharide matrix of the endophyte *Serendipita indica*, its composition and its role as putative harbor for beta-glucan-derived immunity elicitors.

Host manipulation by phloem-limited bacteria

Gitta Coaker

One Shields Avenue, Department of Plant Pathology, University of California, Davis
Correspondence to: gcoaker@ucdavis.edu

Huanglongbing (HLB) is currently the most devastating disease of citrus. HLB is a vector-borne disease associated with the phloem-limited bacterium *Candidatus Liberibacter asiaticus* (CLas). In order to gain greater insight into CLas biology and genetic diversity, we have initiated genome sequencing and comparative analyses of HLB-associated bacteria from diverse geographical regions. Our analyses indicate multiple introductions to California. We have also identified conserved CLas proteins likely involved in virulence and bacterial survival and analyzed their expression in their plant host and insect vector. CLas is able to mask multiple immunogenic MAMP epitopes and individual SEC-dependent effectors exhibit differential expression in plants and psyllids. We also identified papain-like cysteine proteases as virulence targets of the SDE1 effector. These data indicate that CLas attempts to evade plant immune perception and differentially expresses effectors for host and vector manipulation.

The hydrolytic battlefield at the plant-pathogen interface

Renier van der Hoorn

Correspondence to: renier.vanderhoorn@plants.ox.ac.uk

Plants secrete various hydrolases upon pathogen challenge. Some of these hydrolases are suppressed during infection, e.g. by pathogen-derived inhibitors. Using activity-based proteomic approaches we identified glycosidases, proteases and other hydrolases in the apoplast of *Nicotiana benthamiana* that are suppressed upon infection with *Pseudomonas syringae*. One of the suppressed glycosidases is BGAL1, a beta-galactosidase that initiates the degradation of the glycan that covers the flagellin rod, thereby initiating the hydrolytic release of flagellin peptides that are recognized by cell surface receptors. BGAL1 is inhibited by a small molecule coined galactosyrin produced by unrelated *P. syringae* strains that carry the galactosyrin biosynthesis gene cluster. In addition to BGAL1, we identified 24 more hydrolases that are suppressed during infection. Infection of agroinfiltrated tissues overexpressing some of these hydrolases demonstrates that they can act in immunity if they overcome their suppression. The approach to study suppressed hydrolases in the apoplast of infected plants uncovers new and important battlefields at the plant-pathogen interface.

Adaptive evolution at a pathogen effector-host target binding interface is associated with host specificity

Adam Bentham

Norwich Research Park, Colney Ln, Norwich NR4 7U, United Kingdom

Correspondence to: adam.bentham@jic.ac.uk

Targeting of host proteins by effectors is essential for pathogen infection. The AVR-Pik effector from the blast fungus *Magnaporthe oryzae* can interact with host small Heavy Metal Associated (sHMA) proteins. Here, we analysed genomes of *M. oryzae* and identified an AVR-Pik-like (AVR-PikL) family of effectors with sequence similarity to AVR-Pik. We predicted a conserved HMA binding site with only a few, lineage-specific polymorphisms. We hypothesise these AVR-PikL family polymorphisms reflect adaptation to different sHMA proteins across grass hosts. To test our hypothesis, we focussed on AVR-PikL2 which is conserved in all host specific lineages of *M. oryzae*. A yeast 2-hybrid screen of AVR-PikL2/sHMA interactions revealed a broader sHMA binding spectrum for AVR-PikL2_Ta from wheat-infecting *M. oryzae* isolates, compared to the AVR-PikL2_Os allele from rice-infecting isolates. To better understand the nature of these interactions, we solved crystal structures of AVR-PikL2 bound to sHMA proteins. We then generated mutations in the HMA-binding interface of the effector alleles and assessed the effect on binding to a sHMA protein via isothermal titration calorimetry. Together, our data show a single polymorphism at the HMA binding interface of AVR-PikL2_Os and Ta alleles is important for differential binding of a sHMA protein, and may reflect evolutionary adaptation in the effector that emerged after host jumps and specialisation to new effector targets across different host species.

The receptor of the apoplastic effector SCR74 of *Phytophthora infestans* is mapped to a receptor-like kinase cluster in *Solanum microdontum*.

Tatiana Marti Ferrando, Xiao Lin, Richard G.F. Visser, Vivianne G. A. A. Vleeshouwers

Plant Breeding, Wageningen University and Research, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands

Correspondence to: tatiana.martiferrando@wur.nl

Potato is the third most important food crop in the world. Unfortunately, potato suffers from many diseases including the devastating late blight disease, which is caused by the oomycete *Phytophthora infestans*. Nowadays, this pathogen remains problematic despite the breeding efforts to integrate resistance into cultivated potato. Research has been focused on major resistance (R) genes, which can recognize cytoplasmic avirulence (Avr) effectors that exhibit high rates of evolution. R genes are quickly defeated by evolving *P. infestans* races, and for this reason, we need to explore new sources of resistance. Here we target pattern recognition receptors (PRRs) that are mainly receptor-like proteins (RLPs) and receptor-like kinases (RLKs), which recognize apoplastic effectors. We focus on a large, highly polymorphic family of small cysteine-rich (SCR) apoplastic effectors named SCR74 from *P. infestans*. SCR74 is present in at least one copy in all *P. infestans* strains tested. We aim at isolating the PRR that recognizes SCR74 variants in *Solanum microdontum* spp. *gigantophyllum*. Preliminary results showed that the response to this effector is localizing to an RLK cluster. Cloning of this receptor aims to gain more insight into the basal immune response among *Solanum* species. Further studies will include engineering the receptor to recognize other variants from the SCR74 family to generate an even broader and more durable resistance to late blight.

Susceptibility Genes as Targets for Disease Resistance Breeding

Guido Van den Ackerveken

Plant-Microbe Interactions, Department of Biology, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands

Correspondence to: G.vandenackerveken@uu.nl

Resistance traits that are more commonly used in breeding disease resistant crops are genetically dominant and belong mostly to the class of NB-LRR genes that encode receptor proteins detecting pathogen molecules or activities. In contrast, recessive forms of resistance block pathogens in other ways. These resistances are the result of mutation or inactivation of dominant susceptibility genes. Loss of susceptibility traits have been well described for resistance to viruses, and to a lesser extent also bacteria and fungi. The molecular mechanisms by which susceptibility is lost can be very diverse, from failure of pathogen multiplication, and disturbance of feeding relations, to the loss of negative regulation of immunity. Arabidopsis DOWNY MILDEW RESISTANCE 6 is such a susceptibility gene encoding a negative regulator of immunity. In wild type plant, the DMR6-encoded oxygenase hydroxylates salicylic acid (SA) thereby reducing the level of this defense hormone. Mutants defective in DMR6 accumulate SA leading to an enhanced immunity phenotype and disease resistance. If even higher levels of SA accumulate, it goes at the expense of growth. This growth-immunity trade off is of interest, because uncoupling it would enable combining strong disease resistance while maintaining optimal growth. In my presentation I will discuss our research aimed at uncoupling the trade off and how we can utilize susceptibility genes as targets for breeding.

Protein modifier SUMO recruits E2 conjugating enzyme (SCE1) to the Replication initiator protein to allow replication of the Geminivirus TYLCV

Francesca Maio, Manuel Arroyo-Mateosa, Mark Kwaaitaal, Eduardo R Bejarano, Marcel Prins and **Harrold A van den Burg**¹

¹ Molecular Plant Pathology, Swammerdam Institute for Life Sciences (SILS), University of Amsterdam, Science
Correspondence to: h.a.vandenburg@uva.nl

Geminiviruses are small ssDNA viruses that infect a wide range of plants. In order to create a cellular environment favorable for viral replication, geminiviruses manipulate the plant cell cycle. We study the role of the viral Replication initiator protein (Rep, AL1, AC1) during reprogramming of the cell cycle and subsequent DNA replication. Research by numerous groups demonstrated that Rep interacts with a plethora of host factors including the SUMO E2 conjugation enzyme 1 (SCE1) and PCNA. We recently reported that Lys residues in the N-terminal half of Rep are required for nuclear localization of Rep from Tomato Yellow Leaf Curl Virus (TYLCV) (Maio et al., 2019). Strikingly, the same residues are essential for Rep from Tomato Golden Mosaic Virus (TGMV) to interact with SCE1. This interaction with Rep appears to suppress SUMO conjugation at a critical residue for PCNA function (Lys164) (Arroyo-Mateos et al., 2018). I will present data that Rep also interacts directly with the protein modifier SUMO via a novel peptide motif; this motif is widely conserved across Geminivirus genomes. Mutating this SUMO interacting motif (SIM) not only prevents Rep from interacting with SUMO, but it also hinders the interaction with SCE1 while also blocking viral replication. Moreover, these three proteins localize together in nuclear bodies, which we previously defined to be sites of SUMO conjugation activity (Mazur et al., 2019). Our data thus suggest that Rep recruits both SCE1 and SUMO to manipulate yet another nuclear function essential for viral DNA replication by stimulating SUMO modification of an unknown protein without altering the global sumoylation profile.

Protein engineering expands the effector recognition profile of a plant NLR immune receptor

Juan Carlos De la Concepcion¹, Franceschetti, M.¹, Terauchi, R.^{2,3}, Kamoun, S.⁴ and Banfield M.¹

¹Department of Biological Chemistry, John Innes Centre, Norwich Research Park, Norwich, UK

²Laboratory of Crop Evolution, Graduate School of Agriculture, Kyoto University, Kyoto, Japan

³Division of Genomics and Breeding, Iwate Biotechnology Research Centre, Iwate, Japan

⁴The Sainsbury Laboratory, Norwich Research Park, Norwich, UK

Correspondence to: juan.de-la-concepcion@jic.ac.uk

Ever since their discovery, plant immune receptors of the NLR (Nucleotide-Binding Leucine-Rich Repeats) superfamily have been the focus of biotechnological efforts to expand their activities and enhance resistance to diverse diseases. However, this has proven challenging to date, in part due to their narrow response specificity and because their activation mechanisms are still not fully understood. Here, we used structure-guided protein engineering to expand the response profile of the rice NLR Pik to variants of the rice blast pathogen effector AVR-Pik. We first revealed the structural basis of effector recognition by a Heavy Metal Associated (HMA) domain integrated in the sensor NLR Pik-1. Then, we modified the effector-binding interface of Pik-HMA, increasing the binding affinity for the AVR-Pik effectors *in vivo* and *in vitro*. This translated to a gain of immune response to previously unrecognized AVR-Pik effector in planta. Our results provide a proof-of-concept for the use of structural biology to guide engineering of plant immune receptors. This strategy can be applied to enhance pathogen recognition in crops, potentially achieving resistance to pathogen strains that are not currently recognized in nature.

Allelopathic plant-plant interactions: the additional mechanism of the dominance of invasive Japanese and Bohemian knotweed

Katarina Šoln, Jasna Dolenc Koce

University of Ljubljana, Biotechnical faculty, Dept. of Biology, Večna pot 111, SI-1000 Ljubljana, Slovenia

Corresponding author: katarina.soln@bf.uni-lj.si

In plant-plant interactions allelopathy plays an important role. Invasive plants such as Japanese knotweed (*Fallopia japonica*) and Bohemian knotweed (*F. xbohemica*) release allelopathic compounds in the soil and suppress the growth of potential plant competitors. The aim of our study was to evaluate the effect of methanol rhizome extracts of Japanese and Bohemian knotweed on the early growth of radish (*Raphanus sativus*). After three days, morphological and biochemical characteristics of radish were examined. Germination of radish seeds was delayed due to the exposure to the extracts, but mainly not inhibited. Our result showed that knotweed extracts affected especially the primary root of treated plants. Both knotweed taxa reduced root growth and mainly had no impact on shoot height. The effect was concentration-dependent. Staining with diaminobenzidine showed increased accumulation of hydrogen peroxide in roots. Also, after the exposure to knotweed extracts, total antioxidative capacity and lipid peroxidation in radish roots increased.

Understanding and exploiting immune receptors in wheat and their wild relatives

Brande Wulff

John Innes Centre, Norwich Research Park, Norwich, United Kingdom

Correspondence to: brande.wulff@jic.ac.uk

Genetic diversity for disease resistance has been fragmented and eroded in bread wheat through polyploidisation, domestication and breeding. We have developed sequence-configured panels of wheat and wild progenitors for high-throughput identification of disease resistance genes by association mapping. Cloned resistance genes can speed up resistance breeding through generation of GM stacks or by conventional marker-assisted selection. We propose an internationally coordinated effort to generate a wheat resistance gene atlas to facilitate more judicious deployment of resistance genes in GM and conventional breeding programmes.

Effector-driven breeding for disease resistance in potato

Vivianne G. A. A. Vleeshouwers

Plant Breeding, Wageningen University & Research, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands.

Correspondence to: vivianne.vleeshouwers@wur.nl

The world's food security crop potato (*Solanum tuberosum*) is threatened by devastating pathogens such as *Phytophthora infestans* and *Alternaria solani*, which cause late blight and early blight, respectively. Fortunately, wild tuber-bearing *Solanum* species have accumulated a diversity of immune receptors during evolution. To identify and characterize these receptors, we are functionally screening effectors in resistant *Solanum* germplasm for mounting defense responses. While breeding for late blight resistance has so far exclusively relied on nucleotide-binding leucine-rich repeat (NLR) genes, which are typically quickly defeated by fast-evolving cytoplasmic effectors of *P. infestans*, *Solanum* plants also contain pathogen recognition receptors (PRR) that recognize apoplastic effectors. Functional studies with a number of different immune receptors identified so far show that they can contribute to resistance to *P. infestans*. Also for early blight resistance, we initiated effectoromics and are exploring the *Solanum* germplasm. We obtained a gap-less genome of *A. solani* that we used to predicted effectors, and we aim at identifying receptors that mount defense against *Alternaria*. Ultimately, by studying the repertoire of immune receptors that recognize the diversity of pathogen effectors, we aim at getting profound understanding of the molecular interaction of *Solanum* and its pathogens, and apply this for achieving broader and potentially more durable disease resistance.

Copy number variation of genes involved in biotic stress interactions in a model plant *Arabidopsis thaliana*

Agnieszka Żmieńko, Anastasiia Satyr, Paweł Wojciechowski, Aleksandra Świercz

Institute of Bioorganic Chemistry Polish Academy of Sciences, Noskowskiego 12/14, 61-704 Poznan, Poland

Correspondence to: akisiel@ibch.poznan.pl

Examining allelic variation of genes related to biotic stress response is critical to understanding the mechanisms underlying plant innate immunity and their evolution. There is increasing evidence that gene copy number variation (CNV) constitutes an important element of this polymorphism. We previously used whole-genome sequencing data from 1,064 *Arabidopsis thaliana* accessions to create a map of CNVs in this plant (Zmienko et al., in review). The CNVs fully overlap 18.3% protein coding genes (CNV-genes), with the enrichment for evolutionary young ones and genes involved in stress and defense, many of them being multiallelic regarding the copy number. Here we focus on characterizing CNV-genes involved in biotic interactions, in particular on receptor-like protein (RLP) genes and nucleotide binding site-leucine-rich repeat (NBS-LRR) genes. With the support from long-read data analysis and publicly available assemblies of *A. thaliana* accessions, we show extensive structural diversity of these genes. We also demonstrate, by a genome-wide association study on 23 defense-related phenotypes from the Atwell study (Nature 465,2010) that CNVs may serve as powerful and informative markers for traits where copy number polymorphism is a causative agent of observed phenotypic variation.

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Abstracts of Poster Presentations



Poster №1:

Towards understanding of bZIP10 dependent biotic stress responses in *Arabidopsis thaliana*

A. Garg¹, M. Steenbergen², L. Pedrotti³, W. Droege-Laser³, S.C.M. Van Wees², [Christina Chaban](#)¹

¹ZMBP, University of Tuebingen, Germany

²Institute of Environmental biology, Utrecht University, The Netherlands

³Biozentrum, Wuerzburg University, Germany

Correspondence to: christina.chaban@zmbp.uni-tuebingen.de

The plant bZIP transcription factors are involved in the regulation of different cellular processes including responses to biotic stress. We have previously shown that bZIP10 acts as a positive regulator of resistance towards biotrophic fungus *Hyaloperonospora parasitica* (Kaminaka et al., *Embo J*, 2006). To better understand the role of bZIP10 in defense responses, *Arabidopsis* lines ectopically expressing bZIP10 or its dominant negative counterpart were used in assays with hemibiotrophic bacteria, necrotrophic fungus, as well as herbivores. The over-expression of bZIP10 in *Arabidopsis* resulted in enhanced tolerance of the plants towards the biotrophic pathogen *Pseudomonas syringae*, while exhibiting an increased susceptibility against the necrotrophic pathogen *Botrytis cinerea* and the herbivore *Mamestra brassicae*. It seems that bZIP10 plays a positive role in the salicylic acid mediated defence responses against biotrophic pathogens while indirectly antagonizing the jasmonic acid mediated defence responses against the necrotrophs and herbivores, thereby facilitating a cross-talk between SA- and JA-defence pathways in *Arabidopsis thaliana* (Garg, PhD Thesis, Eberhard Karls Universitaet Tuebingen, 2018).

Poster №2:

Functional Characterization of Flagellin and EF-Tu-Responsive Long Noncoding RNAs in *Arabidopsis*

Jimin Lee, Moon-Joo Lee, and [Choonkyun Jung](#)

Graduate School of International Agricultural Technology, Seoul National University, Pyeongchang, Republic of Korea

Correspondence to: jasmin@snu.ac.kr

Long noncoding RNAs (lncRNAs) have emerged as important regulatory factors of diverse biological processes. However, plant lncRNAs involved in innate immunity remain largely unknown. Plant innate immune responses are initiated upon the perception of PAMP (pathogen-associated molecular pattern) such as flagellin (flg22) and EF-Tu (elf18). In this study, we analyzed custom lncRNA array datasets generated from PAMP treatments and identified 1,370 *Arabidopsis* lncRNAs induced or repressed by flg22 and elf18. Real-time RT-PCR validation confirmed the differential expression of these lncRNAs in PAMP and tissue-specific manners. Out of them, we chose 13 ELENAs (elf18-induced long noncoding RNAs) for further analysis. All ELENAs are induced by PAMP treatments, and ELENA5, 7, 11, and 12 are responsive to salicylic acid. ELENA3 is a natural antisense transcript of acyl-transferase induced by PAMP, and ELENA3 knockout plants show decreased callose deposition. ELENA11 is a long intergenic noncoding RNA as a negative regulator, and ELENA11 knockout plants show increased expression of nearby lipase and elevated callose deposition. Our results suggest that ELENAs play key roles in the transcriptional regulation of innate immunity.

Poster №3:

Phenotypic characterization of a mutant impaired in epidermal hair development in tomato

Jae-In Chun¹, Jin-Ho Kang^{1,2}

¹Institute of GreenBio Science and Technology, Seoul National University, Pyeongchang 25354, Korea

²Graduate School of International Agricultural Technology and Crop Biotechnology Institute, Seoul National University, Pyeongchang 25354, Korea

Correspondence to: kangjinho@snu.ac.kr

Trichomes are hair-like structure derived from plant epidermis that acts as a plant defense against biotic and abiotic stresses and are uni- or multicellular structures. Trichomes exist in the wide range of plant species and are classified as either glandular or non-glandular types. Glandular trichomes function in a chemical defense against herbivore, and non-glandular trichomes function as physiological barriers for biotic and environmental stresses. In this study, we characterized a monogenic recessive tomato mutant called no trichome (nt). To analyze the morphology of trichomes on the nt mutant in detail, we observed trichomes with a dissecting microscope and a cryo-SEM. Compared with wild-type plants which have four types of glandular (type I, IV, VI, VII) and three types of non-glandular (type II, III, V) trichomes on the all aerial tissue, the nt mutant does not have all types of trichomes on young stems. In addition, the nt mutant has shorter stems and fewer branches compared with wild-type plants. To identify the NT gene, we are conducting the map-based cloning.

Poster №4:

Towards the identification of *Ralstonia solanacearum* effectors associated with avirulence in Wild tomato relative, *Solanum pimpinellifolium*

Ankita Pandey¹, Ha-yeon Yoon¹, Hayoung Moon¹, Young Kee Lee³, Seungdon Lee³, Heejung Cho³ and Kee Hoon Sohn^{1,2}

¹Department of Life Science, Pohang University of Science and Technology, Pohang, 37673, Republic of Korea

²School of Interdisciplinary Bioscience and Bioengineering, Pohang University of Science and Technology, Pohang 37673, Republic of Korea;

³National Institute of Agricultural Sciences, Rural Development Administration, Jeonju, Republic of Korea

Correspondence to: ankitap03@postech.ac.kr

Petunia hybrida L. is characterized by a Solanaceae type SI in which self pollen is rejected by S-RNase being expressed by pistil as result of its cytotoxic function. Despite numerous attempts to understand the mechanism of S-RNase-based SI it remains just incompletely studied. The involvement of the hormonal regulation in SI mechanism is the finding observed by us for the first time (Kovaleva and Zakharova 2003) and allowed to conclude that ethylene is the factor required for pollen tube growth. Recently we have demonstrated the presence of PCD markers, such as DNA fragmentation, in growing *in vivo* self-incompatible pollen tubes during the passage of the SI mechanism as well as possible involvement of hormonal regulation in the its action (Kovaleva et al. 2019). Preliminary treatment of stigmas with AOA, inhibitor of ACC synthesis, led to stimulation of pollen tubes growth when the latter did not exhibit any hallmarks of PCD. These data argue in favor of assumption that ethylene controls the passage of PCD in incompatible pollen tubes in the course SI functioning.

Poster №5:

Identifying the receptor of Phytophthora Pep-13/25 in Solanum species

Yerisf Torres Ascurra¹, Xiao Lin², Happyka Fillianti¹, Richard R.G.F. Visser¹, Thorsten Nürnberger³, Vivianne G.A.A. Vleeshouwers¹

¹Plant Breeding, Wageningen University and Research

²The Sainsbury Laboratory, University of East Anglia, Norwich Research Park

³Centre for Plant Molecular Biology, Eberhard Karls University of Tübingen

Correspondence to: yerisf.torresascurra@wur.nl

The late blight disease caused by the oomycete pathogen *Phytophthora infestans* is the major threat for potato production. Breeding for resistance to late blight has been focused in the introgression of cytoplasmic resistance (R) genes into potato cultivars. However, these R genes have failed to provide durable resistance to the disease. An alternative source of immunity relies on pattern recognition receptors (PRR) that are located on the cell surface and recognize apoplastic effectors or microbe-associated molecular patterns (MAMPs). One well characterized oomycete MAMP is Pep-13/25, a conserved peptide motif derived from a transglutaminase that is present in all *Phytophthora* species, including *P. infestans*. Infiltrations with Pep-13/25 leads to HR-like cell death responses in various wild *Solanum* species and cultivated potatoes. To identify the PRR of Pep13/25, a genetic mapping approach was conducted and combined with BSR-Seq analyses. This has resulted so far in an 150 kb mapping interval of *Solanum microdontum* based on the DM potato reference genome. Physical maps have been reconstructed and various receptor-like kinases (RLK) candidate genes from *S. microdontum* have been obtained. To accelerate the identification of the receptor, a segregating population of DM was generated in parallel, and the fine mapping resulted in a region of 126 kb. Overall this work aims to identify novel surface immune receptors in potato that will contribute to understand cell surface-triggered immunity. Exploiting the PRR and stacking them with R genes can provide a more profound strategy to achieve a more durable resistance to late blight in potato.

Poster №6:

Genome-wide DNA methylation analysis reveals the changes in flax response to the infection of the pathogenic and non-pathogenic strain of *Fusarium oxysporum*

Wioleta Wojtasik¹, Bartosz Kozak², Anna Kulma¹

¹Department of Genetic Biochemistry, Faculty of Biotechnology, University of Wrocław, Przybyszewskiego 63, 51-148 Wrocław, Poland

²Department of Genetics, Plant Breeding and Seed Production, Faculty of Life Sciences and Technology, Wrocław University of Environmental and Plant Sciences, pl. Grunwaldzki 24A, 50-363 Wrocław, Poland

Correspondence to: wioleta.wojtasik@uwr.edu.pl

The most dangerous flax pathogen is *Fusarium oxysporum* sp. *linii*, the infection by which leads to fusarium wilt of flax, thereby reducing the yield by up to 20%. It is now known that the non-pathogenic strain of *Fusarium oxysporum* may limit the development of plant diseases, but the exact mechanism of its action is still unknown. It is thought to work at the level of DNA methylation. Therefore, the goal of our research was to analyze DNA methylation in flax seedlings treated with the pathogenic and non-pathogenic strain of *Fusarium oxysporum*. Genome-wide mapping of methylated cytosine in flax revealed changes of methylation patterns in different regions of the genes depending on the *F. oxysporum* strain which could indicate the important role of DNA methylation in the process of infection with a pathogenic strain and sensitization of a non-pathogenic strain.

Poster №7:

The non-mevalonate pathway of terpenoid synthesis activation in flax upon *Fusarium oxysporum* infection results in increased ABA synthesis

Aleksandra Boba¹, Kamil Kostyn², Anna Kulma¹

¹Faculty of Biotechnology, University of Wrocław, Przybyszewskiego 63/77, 51-148 Wrocław, Poland

²Department of Genetics, Plant Breeding and Seed Production, Faculty of Life Sciences and Technology, Wrocław University of Environmental and Plant Sciences, Plac Grunwaldzki 24A, 53-363 Wrocław, Poland

Correspondence to: aleksandra.boba@uwr.edu.pl

Plants have developed a number of defense strategies against the adverse effects of fungi such as *Fusarium oxysporum*. One such defense is the production of antioxidant secondary metabolites, which fall into two main groups: the phenylpropanoids and the terpenoids (sterols, carotenoids, tocopherols). Functions of phenylpropanoids and the activity of the genes involved in these compounds' synthesis during pathogen infection have been extensively studied. However, neither the functions of the isoprenoids, nor the expression of genes of the terpenoid biosynthesis after pathogen attack are clear. In order to broaden our knowledge of isoprenoid production and function in response to *Fusarium oxysporum* attack in flax, we investigated the changes in expressions of several genes involved in terpenoid synthesis triggered by pathogen attack. We discovered that in flax the non-mevalonate (MEP) pathway is strongly activated after pathogen infection. Changes of gene expression in the terpenoid pathway, especially of those connected with ABA synthesis are correlated with increased ABA level. As confirmed by our staining experiments, ABA may be connected with the early responses of flax to *F. oxysporum* infection.

Poster №8:

Gene duplication and mutation in the emergence of a novel aggressive allele of the AVR-Pik effector in the rice blast fungus

Apinya, Longya^{1,2}, Chaivarakun, C.¹, Marina F.², Josephine, H.R.M.², Mark, J.B.² and Chatchawan, J.¹

¹Department of Genetics, Faculty of Science, Kasetsart University, Bangkok, 10900, Thailand

²Department of Biological Chemistry, John Innes Centre, Norwich, NR4 7UH, UK

Correspondence to: apinya.longya@gmail.com

Rice blast disease, caused by *Magnaporthe oryzae*, is the most devastating disease affecting rice worldwide. In the field, resistant varieties are unstable and can become susceptible to disease within a few years of release due to the adaptive potential of the blast fungus, specifically in the effector gene pool. Here, we analyzed genetic variation of the effector gene AVR-Pik in 58 rice blast isolates from Thailand and examined the interaction between AVR-Pik and the cognate rice resistance gene Pik. Our results reveal that Thai rice blast isolates are very diverse. We observe four AVR-Pik variants in the population, including 3 previously identified variants, AVR-PikA, -D, and -E, and one novel aggressive allele which we named AVR-PikF. Interestingly, 28 of the isolates contained two copies of AVR-Pik, this was validated by quantitative PCR and Southern blot analysis. Both genes were found to be expressed. Resequencing of one of the isolates with two copies revealed two different contigs of 705 and 709 nt containing the two alleles. Blast isolates expressing only AVR-PikF show high virulence to rice cultivars encoding allelic Pik resistance genes, and the AVR-PikF protein does not interact with the integrated HMA of the Pik resistance protein in vitro by gel filtration assay, AVR-PikF allele differs from AVR-PikA by M78K mutation which is located in the interface between AVR-PikF and Pikm-HMA, and this change may have disrupted interaction between them, suggesting a mechanism for immune evasion.

Poster №9:

Identifying apoplastic plant proteins targeted by plant pathogens

Felix Homma

Department of Plant Sciences, S Parks Rd, Oxford OX1 3RB, United Kingdom

Correspondence to: Felix.Homma@plants.ox.ac.uk

Yield losses due to plant pathogens pose a substantial threat to agriculture. A decisive parameter for the susceptibility of a plant to a particular pathogen is the defense response in the plant apoplast (intercellular space). Apoplastic immune responses include the secretion of different proteins, many of which are hydrolases (eg. proteases, glycosidases, lipases). It is expected that some of these secreted immune proteins are targeted by pathogen-derived proteins. These proteins are under evolutionary pressure to evade antagonistic interactions. We aim to characterise and compare immuno secretomes of different Solanum species to identify apoplastic proteins that are involved in plant-pathogen interactions. This involves combining a proteomic approach to define immuno secretomes with computational analyses for traces of positive selection. Recent genome resequencing projects of different Solanum species resulted in a genomic resource of over 500 genomes which makes this combined approach feasible. Further characterisation of identified proteins includes the quantification of their contribution to biotic stress tolerance. This novel approach will improve our understanding of plant immunity and contribute to sustainable solutions of reducing yield losses in agriculture.

Poster №10:

SnRK1-mediated tolerance to clubroot infection in Arabidopsis thaliana

Harshavardhanan Vijayakumar¹, Heba Ibrahim², Barbara De Coninck², Filip Rolland¹

¹Molecular Plant Biology, Biology Department, KU Leuven, Kasteelpark Arenberg 31, 3001 Heverlee, Belgium

²Division of Crop Biotechnics, Department of Biosystems, KU Leuven, Willem de Croylaan 42, 3001 Leuven, Belgium

Correspondence to: vharshavardhanan@gmail.com

Plasmodiophora brassicae is a soil-borne biotrophic pathogen, causing clubroot disease in brassica species with substantial economic losses. Infection occurs via root hairs where the pathogen proliferates and first symptoms appear once the pathogen migrates to root cortex tissues causing gall formation, producing a strong metabolic sink interfering with water and nutrient uptake, eventually leading to death. Gall formation are associated with distinct transcriptional and physiological reprogramming, but more research is required to understand the specific mechanisms involved. The SnRK1 kinase is a highly conserved cellular energy sensor, which is activated upon carbon depletion. In this project, we investigate the effect of SnRK1 activity on P. brassicae infection and proliferation in A. thaliana using a simple hydroponic system. Infection assays with SnRK1 transgenic lines and metabolic, enzymatic and gene expression analyses at different infection stages are a first step in understanding how SnRK1 modification can contribute in sustainable resistance against this important disease.

Poster №11:

The effect of *Debaryomyces hansenii* on changes in the expression of genes involved in the defense responses of *Triticum turgidum* ssp. *durum* during *Fusarium graminearum* infection

Urszula Wachowska¹, Wioletta Pluskota², Margaret Balcerzak³, Tomasz Kurowski¹

^{1,2}University of Warmia and Mazury

¹Department of Entomology, Phytopathology and Molecular Diagnostics

²Department of Physiology, Genetics and Biotechnology, Poland

³Ottawa Research and Development Centre, Agriculture and Agri-Food, Canada

Correspondence to: urszula.wachowska@uwm.edu.pl

Durum wheat (*Triticum turgidum* ssp. *durum*) is extremely susceptible to *Fusarium* head blight (FHB). Biocontrol agents such as yeasts, including *Debaryomyces hansenii*, used to suppress *F. graminearum*, could pose a viable alternative to fungicides. The aim of this study was to evaluate the expression of the gene encoding chitinase in *Triticum turgidum* ssp. *durum* during infection with *F. graminearum* after biocontrol treatment. The mRNA transcript levels were normalized based on the mRNA transcript levels of the gene encoding glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and RNA-binding heterogeneous nuclear ribonucleoprotein Q (hnRNPQ). The expression of the chitinase gene increased 6-fold between the first and second day after spike inoculation with the spore suspension of *F. graminearum*. The biocontrol treatment that involved the application of yeast cell suspension to wheat spikes contributed to a 14-fold increase in chitinase gene expression between 24 and 48 hours of *F. graminearum* infection. The biocontrol treatment exerted an inhibitory effect on the expression of selected genes involved in the defense responses of durum wheat during *F. graminearum* infection.

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List of Participants

Hannah Bemm

KWS Saat Se & Co. KgaA
Einbeck, Germany
hannah.bemm@kws.com

Pierre Buscaill

University Of Oxford
Department Of Plant Sciences
Oxford, United Kingdom
pierre.buscaill@plants.ox.ac.uk

Gitta Coaker

University Of California, Davis
Plant Pathology
Davis, United States
glcoaker@ucdavis.edu

Farid El Kasmi

Eberhard Karls University Of Tübingen
ZMBP - Center For Plant Molecular
Biology
Tübingen, Germany
farid.el-kasmi@zmbp.uni-tuebingen.de

Felix Homma

University Of Oxford
Department Of Plant Sciences
Oxford, United Kingdom
felix.homma@plants.ox.ac.uk

Jin Ho Kang

Seoul National University
Pyeongchang, South Korea
kangjinho@snu.ac.kr

Bostjan Kobe

University Of Queensland
School Of Chemistry And Molecular
Biosciences
Brisbane, Australia
b.kobe@uq.edu.au

Seungeun Lee

Yonsei University
Seoul, South Korea
seungeun95@yonsei.ac.kr

Adam Bentham

John Innes Centre
Biological Chemistry
Norwich, United Kingdom
adam.bentham@jic.ac.uk

Christina Chaban

Tuebingen University
Tuebingen, Germany
christina.chaban@zmbp.uni-
tuebingen.de

Juan Carlos De La Concepcion

John Innes Centre
Norwich, United Kingdom
juan.de-la-concepcion@jic.ac.uk

Niko Geldner

University Of Lausanne
Lausanne, Switzerland
Niko.Geldner@unil.ch

Laura Jaakola

Uit The Arctic University Of Norway
Department Of Arctic And Marine
Biology
Tromso, Norway
laura.jaakola@uit.no

Šoln Katarina

University Of Ljubljana
Biotechnical Faculty, Dept. Of Biology
Ljubljana, Slovenia
katarina.soln@gmail.com

Thomas Kroj

Inrae
Laboratory Of Biology And Genetics Of
Plant Pathogen Interactions
Montpellier, France
thomas.kroj@inra.fr

Apinya Longya

Kasetsart University
Department of Genetics
Bangkok, Thailand
apinya.longya@gmail.com

Aleksandra Boba

University Of Wroclaw
Faculty Of Biotechnology
Wroclaw, Poland
Aleksandra.boba@uwr.edu.pl

Nahyun Cho

Yonsei University
Seoul, South Korea
nhcho93@yonsei.ac.kr

Manos Domazakis

Enza Zaden
Enkhuizen, Netherlands
m.domazakis@enzazaden.nl

Karin Hage-Ahmed

University of Natural Resources and
Life Sciences, Vienna
Institute of Plant Protection
Tulln, Austria
karin.hageahmed@boku.ac.at

Choonkyun Jung

Seoul National University
Department of International
Agricultural Technology
Pyeongchang, South Korea
jasmin@snu.ac.kr

Boyoung Kim

Seoul National University
Seoul, South Korea
hihi4823@gmail.com

Anna Kulma

University Of Wroclaw
Faculty Of Biotechnology
Wroclaw, Poland
anna.kulma@uwr.edu.pl

Takaki Maekawa

Max Planck Institute For Plant
Breeding Research
Cologne, Germany
maekawa@mpipz.mpg.de

Tatiana Marti Ferrando
Wageningen University & Research
Plant Breeding
Wageningen, Netherlands
tatiana.martiferrando@wur.nl

Andrea Romero Perez
University Of Ghent
Biotechnology
Gent, Belgium
Andrea.RomeroPerez@UGent.be

Guido Sessa
Tel Aviv University
School of Plant Sciences
Tel Aviv, Israel
guidos@tauex.tau.ac.il

Frank Takken
University Of Amsterdam
Sils -Molecular Plant Pathology
Amsterdam, Netherlands
f.l.w.takken@uva.nl

Yerisf Torres Ascurra
Wageningen University & Research
Plant Breeding
Wageningen, Netherlands
yerisf.torresascurra@wur.nl

Harrold Van den Burg
University of Amsterdam
Molecular Plant Pathology
Amsterdam, Netherlands
h.a.vandenburg@uva.nl

Vivianne Vleeshouwers
Wageningen University
Wageningen, Netherlands
vivianne.vleeshouwers@wur.nl

Alan Wanke
University of Cologne
Institute for Plant Sciences
Köln, Germany
alan.wanke@uni-koeln.de

Brande Wulff
John Innes Centre
Crop Genetics
Norwich, United Kingdom
brande.wulff@jic.ac.uk

Ankita Pandey
Postech Department Of Life Sciences
Pohang, South Korea
ankitap03@postech.ac.kr

Jennifer Sales
Helmholtz Zentrum Muenchen
Institute Of Biochemical Plant Pathology
Oberschleissheim, Germany
jennifer.sales@helmholtz-muenchen.de

Kee Hoon Sohn
POSTECH
Life Sciences
Pohang, South Korea
khsohn@postech.ac.kr

Wladimir Tameling
Keygene NV
Wageningen, Netherlands
sa@keygene.com

Olha Tyvylik
Perspektiva Holding Llc
Kyiv, Ukraine
natalka.grush@gmail.com

Renier Van Der Hoorn
University Of Oxford
Department Of Plant Sciences
Oxford, United Kingdom
renier.vanderhoorn@plants.ox.ac.uk

Corina Vlot-Schuster
Helmholtz Zentrum Muenchen
Institute Of Biochemical Plant Pathology
Neuherberg, Germany
corina.vlot@helmholtz-muenchen.de

Wioleta Wojtasik-Górna
University Of Wroclaw
Wroclaw, Poland
wioleta.wojtasik@uwr.edu.pl

Seong Gwan Yu
Yonsei University
Department Of Systems Biology
Seoul, South Korea
sgyu@yonsei.ac.kr

Agnieszka Żmieńko
ICHB PAN
Poznan, Poland
akisiel@ibch.poznan.pl

David Percival
Dalhousie University
Plant, Food, and Environmental
Sciences
Halifax, Canada
David.Percival@dal.ca

Sebastian Schornack
University Of Cambridge
Sainsbury Laboratory
Cambridge, United Kingdom
sebastian.schornack@slcu.cam.ac.uk

Steven Spoel
University Of Edinburgh
Institute Of Molecular Plant Sciences
Edinburgh, United Kingdom
steven.spoel@ed.ac.uk

Kathrin Thor
The Sainsbury Laboratory
Norwich, United Kingdom
kathrin.thor@tsl.ac.uk

Guido Van Den Ackerveken
Utrecht University
Department Of Biology
Utrecht, Netherlands
g.vandenackerveken@uu.nl

Harsha Vardhanan Vijayakumar
Ku Leuven
Institute Of Botany And Microbiology
Heverlee, Belgium
vharshavardhanan@gmail.com

Urszula Wachowska
University of Warmia And Mazury In
Olsztyn
Olsztyn, Poland
urszula.wachowska@uwm.edu.pl

Michael Wrzaczek
University Of Helsinki
Faculty Of Biological And
Environmental Sciences
Helsinki, Finland
michael.wrzaczek@helsinki.fi

Alga Zuccaro
University Of Cologne
Institute For Plant Sciences
Cologne, Germany
azuccaro@uni-koeln.de

