Meetings

A snapshot of molecular plant–microbe interaction research

XVI International Congress on Molecular Plant–Microbe Interactions, Rhodes, Greece, July 2014

Plants and microbes are in a continuous arms race to maintain their predominance within their particular niche. Understanding the complexity of these plant–microbe interactions is of utmost importance as it can provide new insights into the mechanisms mediating disease processes and in turn inspire new plant breeding strategies. The International Society for Molecular Plant–Microbe Interactions (IS-MPMI) invited scientists from around the world to share their findings during the XVI International Congress on Molecular Plant–Microbe Interactions, which was held on the beautiful island of Rhodes in Greece (Fig. 1). The congress was organized by the Agricultural University of Athens, the Hellenic Phytopathology Society, and the Hellenic Society of Phytiatry and provided over 1100 participants from 55 countries with the opportunity to present and discuss their current and future research. A great number of talks and posters were presented, however our aim within this report is to provide a snapshot of the discipline by focusing on just some of the exciting research and discussions which took place. The key topics discussed were virulence factors, epigenetic regulation, hormones, symbiosis factors, toxins, signaling pathways, microbe recognition, immunity, and pathogen diagnostics. Effector biology was also a recurrent theme in many plenary and concurrent sessions, indicating the importance of a topic that was also highlighted recently by a Virtual Special Issue in *New Phytologist* (see Kuhn & Panstruga, 2014). In addition to this, throughout the meeting next generation sequencing (NGS) techniques were described and shown to be shedding new light on long-standing issues in microbial ecology.

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Rethinking the plant microbiome

The microbiome, defined as the community of microbes associated with and living in plant tissues, is now under intensive investigation to improve our understanding of its well-known strong effects on plant development, health and resistance to parasites. NGS methods are being applied by different research groups to help understand how the microbiome is colonizing the rhizosphere, the phyllosphere and the root and leaf intercellular compartment, that is, the endosphere. Soil microbial diversity has often been considered to be critical to the integrity, function and long-term sustainability of soil ecosystems and plants, and it has been shown that plants are the main factor responsible for determining soil microbial community structure (Broughton & Gross, 2000; Smalla *et al.*, 2001). However, a series of large-scale NGS-based projects are providing a new perspective to the field by deciphering the mechanisms regulating the spatial and temporal dynamics of microbial communities *in planta* (e.g. Links *et al.*, 2014). Accordingly, microbiome screening results of wild-type *Arabidopsis thaliana* plants were described by Jeffrey Dangl (Carolina Center for Genome Sciences, University of North Carolina, Chapel Hill, NC, USA) and Paul Schulze-Lefert, Stephane Hacquard, and Yang Bai (Max Planck Institute for Plant Breeding Research, Köln, Germany; e.g. Lundberg *et al.*, 2012; Bulgarelli *et al.*, 2012; Schlaeppi *et al.*, 2013). The main goal of this research is to identify microbes that help plants by improving growth, resistance to drought, and the ability to fend off pests and diseases for agricultural applications. Comparable analyses were performed by Gerald Tuskan's team (Oak Ridge National Laboratory, TN, USA) using poplar as a model (Shakya *et al.*, 2013).

Thinking outside the effector's box

Exciting new findings on several microbial effectors were discussed in a number of different sessions. HopAO1 from *Pseudomonas syringae* has the potential to block the signaling activity of pattern recognition receptors (PRR) by targeting the downstream tyrosine phosphorylation mechanism (Macho *et al.*, 2014). Further, *P. syringae* effector HopX1 promotes activation of jasmonate signaling by targeting JAZ proteins to facilitate infection (Gimenez-Ibanez *et al.*, 2014). This conference also provided an opportunity for researchers to broaden their understanding of the new models and concepts in this field (Doehlemann & Hemetsberger, 2013; Doehlemann & Requena, 2014). For example, Timothy Friesen (USDA-AR, Bismarck, ND, USA) talked about the *Stagonospora nodorum*–wheat pathosystem and the SnTox1 effector, a 117 amino acid cysteine-rich protein interacting with the wheat sensitivity/susceptibility protein Snn1. This light-dependent interaction triggers classical defense responses (e.g. oxidative burst, up-regulation of pathogenesis-related proteins) but, interestingly, the recognition of SnTox1 by Snn1 led to susceptibility rather than resistance. They finally showed that this effector is involved in protection against host chitinases (Liu *et al.*, 2012). Natalia Requena (Karlsruhe Institute of Technology, Germany) reported some recent advances on the mechanisms

driving the early steps of the arbuscular mycorrhizal symbiosis. Natalia Requena's team first identified and analyzed a 7 kDa small secreted protein, named SP7 (Secreted Protein 7), required for arbuscular mycorrhiza symbiosis (Kloppholz *et al.*, 2011) which interacts with the pathogenesis-related transcription factor ERF19. Thanks to the recently released *Rhizophagus irregularis* genome (Tisserant *et al.*, 2013), her group has now identified several other effector candidates, some of which share similarities with Crinkler's effectors, such as the ability to induce cell-death on infiltrated leaves. Francis Martin (INRA, Nancy, France) described the most recent results obtained on the mycorrhiza-induced small secreted protein MiSSP7, secreted by the ectomycorrhizal fungus *Laccaria bicolor*. MiSSP7 was found to enter host cells and to localize to the nucleus where it altered host cell transcription. MiSSP7 interacts with the transcriptional repressor protein PtJAZ6 in the nuclei of the host plant *Populus trichocarpa*, where it protects PtJAZ6 from JA-induced degradation (Plett *et al.*, 2014). Furthermore, MiSSP7 is able to counter the negative impacts of JA on fungal colonization of host tissues by repression of JA-induced gene transcription, likely through its interaction with JAZ proteins. These results on the arbuscular and ectomycorrhizal symbioses further the concept that, like pathogenic organisms, mutualistic fungi use effectors to target plant host hormone pathways to foster fungal colonization. Another highlight regarding ectomycorrhizal symbiosis was again the work of Gerald Tuskan's team (Oak Ridge National Laboratory). Using genomics techniques they identified a single gene of poplar (encoding a manno-protein receptor) which functions as a determinant between mycorrhizal and nonmycorrhizal lifestyle/behavior. Moreover, transformation of *A. thaliana* with the respective poplar gene was sufficient to induce a Hartig net-like structure in this nonectomycorrhizal plant.

The IS-MPMI meeting was also a perfect platform to share new results on effectors from unusual models. Saskia Hogenhout (John Innes Centre, Norwich, UK) presented research on the SAP54 effector secreted by the insect transmitted bacteria *Phytoplasma* (Weintraub & Beanland, 2006; Hogenhout *et al.*, 2008). Her team demonstrated that the SAP54 effector destabilizes MADS domain transcription factors (MTF) through interaction with RAD23C and RAD23D proteins in *A. thaliana*. The effects are the formation of leaf-like flowers and increased attractiveness to the *Phytoplasma* vector, a leafhopper. Markus Albert (Centre for Plant Molecular Biology, University of Tübingen, Germany) presented his work on the holoparasitic plant *Cuscuta reflexa* (Fig. 2). This plant infects a broad range of dicotyledonous plant species, except *Solanum lycopersicum*. His group has identified a 2 kDa glycopeptide of *C. reflexa* that triggers defense responses in *S. lycopersicum* but not in susceptible plants. This ParAMP (parasite associated molecular pattern) is detected in nonhost plants through a leucine rich repeat (LRR)-like receptor protein, termed Cuscuta Receptor 1 (CuRe1), inducing defense responses that prevent successful colonization.

Function and engineering of plant recognition receptors

Plant innate immunity is based on the recognition of microbe-associated molecular patterns (MAMPs) by PRR. Identification of new PRRs at the protein level is relatively ineffective due to their low abundance and hydrophobicity. Patrick Boyle (Boyce Thompson Institute for Plant Research, Ithaca, NY, USA) presented a new method to solve this problem called the 5C strategy. First the MAMP-probe is cross-linked to its cognate PRR by UV irradiation and both are then dissolved from the membrane.

Fig. 1 *Hibiscus rosa-sinensis* (Malvaceae) on the island of Rhodes; photographed by Sebastian Wittulsky during the IS-MPMI Congress.

Fig. 2 *Cuscuta reflexa* (Convolvulaceae) during infection of *Solenostemon scutellarioides* (Lamiaceae); photograph kindly provided by Markus Albert, Center for Plant Molecular Biology of Tübingen, Germany.

Click chemistry is applied to add a cleavable isotope-coded affinity purification tag (ICAT). Afterwards the tagged complex is captured and enriched before chemical cleavage. The purified material retains a portion of the ICAT, which facilitates characterization by MS. To demonstrate the effectiveness of the strategy he cross-linked the MAMP flg22 (a peptide derived from the bacterial flagellum protein flagellin) to its PRR FLS2 and purified. This approach promises to help advance the identification of new PRRs and may also provide a system for detecting unknown interacting molecules of proteins of interest.

Georg Felix (Centre for Plant Molecular Biology, University of Tübingen, Germany) described the LRR-receptor kinases FLS2 and EFR of *Arabidopsis* that detect the bacterial proteins flagellin and EF-Tu. He discussed a method based on chimeric variants of such receptors as tool for functional studies (Albert & Felix, 2010; Albert *et al.*, 2010). If the cytoplasmic domains and the SERK co-receptors were reciprocally swapped, the artificial receptors displayed somewhat predictable activities. This research raises the question if this 'two-hybrid-receptor' approach would allow us to trigger a 'chosen' cell response in the future. An artificial triggering system for cell responses such as this could act as a screening tool to select molecules or a desired cell answer in the presence of the cognate ligand.

Manipulation of fungi by plant viruses

Virus-induced gene silencing (VIGS) is a common tool in plant biology used to study the function of genes through transient silencing (Lu *et al.*, 2003). Peter Palukaitis (Seoul Women's University, South Korea) and Donato Gallitelli (Università degli Studi di Bari Aldo Moro, Italy) demonstrated the potential of VIGS in filamentous fungi by showing that several plant viruses have the potential to infect and replicate in *Colletotrichum* or *Phytophthora* species. Further, genetically modified viruses can be used for strong expression of foreign genes (e.g. encoding Green Fluorescent Protein; GFP) or for gene silencing (Mascia *et al.*, 2014). This technique could be utilized in the future to manipulate un-transformable fungi such as biotrophic fungi (e.g. rusts or endosymbionts), as the technique is fast and very powerful.

Diagnosis and diagnostics of plant–microbe interactions

A major component within the field of plant–microbe interactions is diagnostics. Maria Lopez (Institut Valencia d'Investigacions Agraries, Spain) explained how diagnosticians are asked to answer the major question 'Who is out there?' She considered the tools available to address this question and provided examples of the use of high throughput technologies for diagnosis such as genomics, transcriptomics, proteomics and metabolomics – so called 'omics era' technology. In a related talk Rick Mumford (The Food & Environment Research Agency, York, UK) emphasized the necessity for the emergence of new and effective tools for fast and accurate field diagnosis. Several speakers described diagnostic approaches which are being developed and utilized by their groups. Georgios Vidalakis (University of California, USA) presented a novel and rapid method to distinguish between infected and non-infected tissue, even in the absence of symptoms. Andrew Armitage (East Malling Research, UK) set the frame for NGS as yet another important tool that could help in the prediction of host specificity. Another diagnostic approach was proposed by Richard Cooper (University of Bath, UK) who described how pathogen-specific effectors can be used as probes for the diagnosis. Diagnosis is an important factor of practice; however, in the words of Martin H. Fischer, as paraphrased by Rick Mumford, 'we should not forget that it is not the end but the beginning of the practice'.

For a long time the translation of basic science into applied practice has been *the* missing link in the study of plant–microbe interactions. At this meeting, the importance of translational research was represented in many talks; for example, Andrew Bent (University of Wisconsin, Madison, WI, USA) proposed that variation in gene copy number could be involved in resistance. The increased focus was exemplified by Cyril Zipfel from The Sainsbury Laboratory (Norwich, UK) who introduced the TSL+ program, which aims to expand on fundamental research from the laboratory and use it with the aim of reducing worldwide losses to crop diseases. It was clear from many of the talks given that in order to engineer broad-spectrum disease resistance both basic and translational research should be followed in parallel.

RNA silencing and epigenetics in plants

David Baulcombe (University of Cambridge, UK) discussed how heritable genetics might be influenced by small interfering RNA-mediated gene silencing. He showed that progenies of *Arabidopsis* plants infected with *Pseudomonas syringae pv. tomato* DC3000 (PstDC3000) exhibit enhanced resistance to the pathogens *Hyaloperonospora arabidopsidis* and PstDC3000. This mechanism was presented as a result of hypomethylated SA-dependent genes. The hypomethylation led to lower gene expression and therefore enhanced resistance (Luna *et al.*, 2012). A similar effect was shown for tissue-culture regenerated rice plants where demethylation of promoters led to decreased expression levels which were stable across generations (Stroud *et al.*, 2013). These results underlie the possible influence of heritable epigenetics in heritable variation within species. Furthermore, this effect could be used for agricultural purposes. Could seed producers infect their plants to get more resistant progenies? If yes, it would contribute to an improved pathogen resistance strategy without the application of chemical agents in the field.

Conclusion

Undoubtedly, many unanswered questions remain within the field of plant–microbe interactions. This congress highlighted some of the exciting research which is being carried out around the world; however, these are only pieces of a puzzle named microbiome, symbiotic countertrade, or plant–pathogen arms race. In nature the distinction between virulence and avirulence often depends on a few genes or, as aptly surmised by Libo Shan (Texas A & M University, College Station, TX, USA), 'Resistance is the rule and disease is the exception'. The MPMI community will continue to

reveal many such mechanisms in the future and we are glad to be part of it.

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