



IS-MPMI
XVIII CONGRESS
July 14–18, 2019
Glasgow, Scotland

IS-MPMI XVIII Congress

Abstracts of Poster Presentations

Abstracts submitted for presentation at IS-MPMI XVIII Congress in Glasgow, Scotland, July 14–18, 2019. The recommended format for citing congress abstracts, using the first abstract below as an example, is as follows:

Li, F., Upadhyaya, N., Schwessinger, B., Sperschneider, J., Matny, O., Raley, C., Miller, M. E., Silverstein, K., Nguyen-Phuc, H., Hirsch, C. D., Visser, B., Pretorius, Z. A., Steffenson, B., Dodds, P. N., and Figueroa, M. 2019. Contribution of a somatic hybridization event to the emergence of the Ug99 lineage of the wheat stem rust pathogen. (Abstr.) *Molecular Plant-Microbe Interactions* 32:S1.1. <https://doi.org/10.1094/MPMI-32-10-S1.1>

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<https://doi.org/10.1094/MPMI-32-10-S1.1>

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Contribution of a somatic hybridization event to the emergence of the Ug99 lineage of the wheat stem rust pathogen

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Stem rust, caused by *Puccinia graminis* f. sp. *tritici* (*Pgt*), is a devastating disease of wheat and barley. The recognition of *Pgt* as a global threat to food security was substantiated by the emergence of races in the Ug99 lineage from Africa, which are virulent to most wheat cultivars in the world. Similar to other rust fungi, *Pgt* is a dikaryotic organism, with two haploid nuclei (karyons) during the infection process on wheat. We developed a new strategy to generate haplotype-phased genome assemblies of *Pgt* using PacBio long reads, to better understand the genomic architecture and evolution of this pathogen. Comparison of a fully haplotype-resolved assembly of Ug99 to two *Pgt* isolates of the race 21 group found in South Africa and Australia provides evidence that *Pgt* Ug99 arose by a somatic hybridization event caused by nuclear exchange during the vegetative phase. Inter-isolate comparisons indicated that one complete haplotype of the race 21 isolates is shared with *Pgt* Ug99. This is

Co infection of OMMV and OLV-1 enhances symptoms and increases both viruses accumulation and viral derived siRNAs in plants

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Previous extensive field surveys in olive orchards have revealed high levels of Olive mild mosaic virus (OMMV) and Olive latent virus 1 (OLV-1), frequently appearing in mixed infections. These viruses belong to genus Alphavirus and their RNA dependent RNA polymerase (RdRp), as well as their p6 and p8 amino acid sequences share over 87% identity. Preliminary studies have shown that co infection of OMMV and OLV-1 is associated to an intensification of symptoms, as well as an increase in transmission efficiency, suggesting a synergistic effect. Single and double infections of OMMV and OLV-1 were obtained through mechanical inoculation of *Nicotiana benthamiana* plants and the second upper leaf from each inoculated plant was collected at different stages and used for quantitative PCR. In this study we found that the co infection of OMMV and OLV-1 causes an exacerbation of symptoms and increases the accumulation of both viruses in *N. benthamiana* plants. High-throughput sequencing of siRNAs from both viruses in singly and co infected plants showed that OMMV and OLV-1 co infection increased the accumulation of siRNAs, mainly of 21 and 22 nt in length, with most non distinguishable between OMMV and OLV-1 siRNAs. Our findings suggest that siRNAs of both viruses have possible roles in the synergistic interaction between OLV-1 and OMMV in *N. benthamiana* plants. Whether a similar situation occurs in olive fields is not yet known and studies are being pursued.

Isolate specific responses to the fungal pathogen *Zymoseptoria tritici* in wheat and *Brachypodium distachyon*

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Zymoseptoria tritici is the causal agent of Septoria tritici Blotch in wheat which threatens wheat yields. Disease assays using the wheat cultivar Longbow revealed that an Irish field isolate displays disease symptoms including chlorosis and cell death, prior to the occurrence of these symptoms with the Dutch reference isolate IPO323. Increased pycnidia formation was also observed at 21 days postinfection (dpi) with the Irish isolate. RNAseq was carried out to compare gene expression between isolates and revealed a subset of differentially expressed small secreted proteins (SSPs). Similar isolate specific responses were found in the symptoms induced when the non-host grass *B. distachyon* was challenged, including cell death, hydrogen peroxide production and defence gene induction. The Irish isolate was found to be more aggressive on both wheat and *B. distachyon* and formed hyphae within *B. distachyon*. Therefore *B. distachyon* may serve as a useful model to study *Z. tritici*.

Host induced gene silencing in *P. pachyrhizi* effectors interfere in virulence of soybean attenuating fungal pathogenicity

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Asian soybean rust, caused by *Phakopsora pachyrhizi* (Pp), is currently the main disease affecting the crop in Brazil, with an estimated cost for its control of US\$ 2.8 billion per crop season. In rusts and other filamentous plant pathogens, haustoria have been shown to secrete effector proteins into their hosts to permit successful completion of their life cycle. The identification and characterization of Pp effectors are expected to provide insight into the mechanisms by which this fungus manipulates soybean to promote infection and elicit Rpp-mediated defense. Pp families of effector have been predicted based on *P. pachyrhizi* transcriptome expressed in planta, and its ability to suppress plant immunity was previously demonstrated. For the functional characterization, effectors candidates able to suppress PAMP and effector-triggered immunity were evaluated by host induced gene silencing (HIGS) bioassays. Fifteen effectors candidates were first tested independently, and five that showed significant reductions in different phenotypic parameters analyzed were also tested combined. Silencing was confirmed by RT-qPCR and effectors acting at initial times after infection (6/12hai) showed a reduction in three of the four phenotypic parameters evaluated when they were tested alone. When combined, four combinations showed reduced infection.

PGPR-mediated modulation of the wheat microbiome

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Plant defenses can be primed upon local contact of naïve plants with plant growth promoting rhizobacteria (PGPR), leading to enhanced resistance against pathogens. Upon germination, however, plant tissues are quickly colonized by seed-borne bacteria. The presence of such microbiome led us to hypothesize that, in addition to the direct PGPR recognition, PGPR-mediated priming may occur through indirect mechanisms, via modulation of the seed-borne microbiome. To test this hypothesis, characterization of the cultivable microbiome in wheat seedlings inoculated with PGPR *Herbaspirillum seropedicae* (RAM10) was carried out. The results showed a significant decrease of the seed-borne bacterial load in the roots and in the medium surrounding the roots of RAM10-inoculated wheat seedlings, when compared to non-inoculated seedlings. Furthermore, bacterial diversity was reduced in RAM-inoculated seedlings, suggesting that RAM10 modulates the composition of the plant microbiome by favoring specific bacteria. Currently we are involved in 1) characterizing the plant growth promoting traits of the identified seed-borne isolates; and 2) determining the potential of RAM10 as an effective priming agent against the wheat pathogen *Pseudomonas syringae* pv. *atropaciens*. These results will allow us to establish the priming effect as the result of a direct PGPR recognition by the plant, a PGPR-mediated modulation of the plant microbiome, or as the combination of both strategies.

Bacterial vesicles: Double agents for plant defense

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