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2021 IS-MPMI Congress: eSymposia Series Abstracts September 15–16 eSymposia: Pathogen-Host Coevolution— Combating Resistance Breaking in Agriculture

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Effects of elevated temperature on hypersensitive resistance of capsicum plants to capsicum chlorosis virus infection

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Capsicum, an important vegetable crop in Australia, is vulnerable to both elevated temperatures and the tospovirus *Capsicum chlorosis virus* (CaCV). Although CaCV-resistant capsicum breeding lines have been established, no studies have investigated how elevated temperatures may affect this resistance in a changing climate. We found that defense responses of CaCV-resistant plants were compromised at high temperature (HT, 35°C) in some plants. Systemic necrotic spots were seen at 10 dpi in 8/14 resistant CaCV-infected plants grown at HT, while systemic leaves of all plants grown at ambient temperature (AT, 25°C) were free of symptoms. To identify miRNAs involved in resistance-breaking at HT, we compared miRNA expression profiles in CaCV- or buffer-inoculated plants grown at HT or AT. A total of 105 known and 83 novel miRNAs were identified from 12 small RNA libraries. The levels of Can-miR408a-3p, Can-miR397-5p, Can-miR397-3p, and Can-miR398b-3p were suppressed by CaCV infection at AT, while they were induced by CaCV infection at HT. Gene ontology enrichment analysis of miRNA target genes indicated that the lignin metabolic pathway, regulated by Can-miR408a-3p and Can-miR397-5p, was involved in the temperature-mediated resistance-breaking response. This study provides first insights into the involvement of miRNAs that may be critical to temperature-sensitive tospovirus resistance.

***Ustilago maydis* secretes the novel virulence-promoting Lep1 effector for hyphal aggregation in plant tumors**

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The biotrophic basidiomycete fungus *Ustilago maydis* causes smut disease in maize. Hallmarks of the disease are characteristic large tumors in which dark pigmented spores are formed. Here, we functionally characterized a novel core effector *lep1* (*late effector protein 1*) which is highly expressed during tumor formation and contributes to virulence. In tumors, *lep1* mutants show attenuated hyphal aggregation, fail to undergo massive late proliferation and produce only few spores. Upon constitutive expression of *lep1*, cell aggregation is induced and the surface of

filamentous colonies display enhanced hydrophobicity, suggesting that Lep1 is a novel surface-active protein. We show that Lep1 is bound to the cell wall of biotrophic hyphae and associates with the repellent protein Rep1 when constitutively expressed in hyphae. From the phenotypes of *lep1*, *rep1* double mutants as well as from strains constitutively expressing either *lep1* or *rep1*, we conclude that Lep1 acts as a novel kind of cell adhesin which functions together with other surface-active proteins to trigger proliferation of diploid hyphae as well as for induction of the morphological changes associated with spore formation.

Genomic plasticity and virulence diversity of *Fusarium oxysporum* f. sp. *vasinfectum* from cotton fields

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Fusarium oxysporum f. sp. *vasinfectum* (*Fov*) causes massive destructive and irreversible devastating losses in cultivated allotetraploid cotton within one month after planting on naturally infected fields. Different races of this pathogen has evolved causing differential race-specific inimical symptoms on cotton and has recently evolved to become extra virulent in its recent emergence in Texas and New Mexico. The genetic and molecular basis for this erratic behavior of *Fov* is poorly understood. Here we sequenced and analyzed large-scale spatial temporal de novo-assembled genomes of 6 *Fov* isolates differing in virulence and origin and the resequencing of 31 additional isolates providing exhaustive insight into *Fov* genome structure, race evolution, expansion, race-specific supernumerary chromosomes, and effector repertoire. It further allowed us to observe both inter and intra-race genomic variations. Whole genome comparative analysis revealed conserved genes among core regions of the genomes but genome expansion and gene duplication at the supernumerary chromosomes which plays significant role in adaptation and evolution. Upland cotton response to differential races of *Fov* targets the root tip and points to an effector triggered immunity involved in the interaction between *Fov* and cotton. These insights are invaluable and provides a grasp of fusarium cotton interactions, pathogenicity, and infection process.

Natural genetic diversity in the potato resistance gene *RB* confers suppression avoidance from *Phytophthora* effector IPI-O4

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RB is a potato gene that provides resistance to a broad spectrum of genotypes of the late blight pathogen *Phytophthora infestans*. *RB* belongs to the CC-NB-LRR (coiled-coil, nucleotide-binding, leucine-rich repeat) class of resistance (R) genes, a major component of the plant immune system. The RB protein detects the presence of Class I and II IPI-O effectors from *P. infestans* to initiate a hypersensitive resistance response, but this activity is suppressed in the presence of the Class III effector IPI-O4. Using natural genetic variation of RB within potato wild relatives, we identified two amino acids in the CC domain that alter interactions needed for suppression of resistance by IPI-O4. We have found that separate modification of these amino acids in RB can diminish or expand the resistance capability of this protein against *P. infestans* in both *Nicotiana benthamiana* and potato. Our results demonstrate that increased knowledge of the molecular mechanisms that determine resistance activation and R protein suppression by effectors can be utilized to tailor-engineer genes with the potential to provide increased durability.

Subspecies-specific domestication of an NLR immune receptor and reconstruction of broad-spectrum disease resistance in rice

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Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*), a vascular-specific bacterial pathogen. The first cloned rice resistance gene to *Xoo*, *Xa21* from wild rice, encodes a receptor-like kinase, which recognizes a PAMP secreted by the *Xoo* strain PXO99A to trigger PTI. However, the *Xa21*-mediated resistance has been broken by many north Asian strains, such as J18 of Korean race I. In this study, we identified a novel NLR *Xa46* that confers resistance to J18. Interestingly, we found that the model reference varieties, Nipponbare and 9311, have lost the functional *Xa46* gene. We further analysis of the 3K rice germplasm and revealed that *Xa46* was derived from wild rice *O. rufipogon*. The gene has been subjected to negative selection and lost in nearly all of japonica rice accessions. The introduction of the gene greatly decreased yield in japonica. In contrast, the functional *Xa46* locus is enriched in indica rice, and did not cause yield penalty in indica rice. We thus propose that *Xa46* is a target of subspecies-specific domestication in rice. More importantly, rice plants containing *Xa21* and *Xa46* that integrate PTI and ETI, conferred resistance to near all strains tested. Therefore, pyramiding of *Xa46* and *Xa21* from wild rice reconstructs PTI and ETI-integrated broad-spectrum disease resistance in modern rice, showing good potential in rice breeding.

Know your enemy—Maize foliar disease survey and yield loss in smallholder farms, South Africa

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Fungal foliar diseases are a major threat to smallholder maize production in countries such as South Africa. We aimed to identify the main foliar fungal diseases and quantify the impact on yield. A three-year survey in the higher rainfall provinces (Eastern Cape and KwaZulu-Natal) revealed that four diseases co-occurred in most fields surveyed. Disease incidence per season ranged from 62-91% for Northern Leaf Blight (NLB), Common Rust, *Phaeosphaeria* Leaf Spot and Gray Leaf Spot, except for the latter that dropped to 2% in a drought year. Disease severity was greatest overall for NLB (*Exserohilum turcicum*). Yield losses due to NLB of three locally grown susceptible hybrids in a controlled field trial ranged from 36-72%, however several other hybrids showed no significant differences in yield between fungicide sprayed and unsprayed plots. A population genetic study of *Cercospora zeina* (GLS) from smallholder (129 isolates) and commercial farms (239 isolates) was conducted based on microsatellite markers. The pathogen was more diverse in smallholder compared to commercial farms (13% vs 32% clonal fraction, respectively). These studies highlight the importance of maize breeding for multiple disease resistance. Farming practices such as monocultures and chemical control may shift the population dynamics of fungal pathogens such as *C. zeina*.

The ancestral chromosome architecture of downy mildew oomycetes

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An ancestral chromosome configuration for downy mildew oomycetes has been determined using high-quality genome assemblies. A 17-chromosome, telomere-to-telomere (T2T) genome assembly of *Peronospora effusa* was generated using PacBio HiFi reads. This was aligned to a genome assembly of *Bremia lactucae*, genetically oriented into 19 chromosome-scale scaffolds. Analysis of single-copy genes revealed extensive synteny between *P. effusa* and *B. lactucae* indicating that these distinct oomycetes share a common 17-chromosome architecture. Phylogenetics determined that the most recent common ancestor of *B. lactucae* and *P. effusa* is common to all downy mildews and several *Phytophthora* species. Therefore this 17-chromosome configuration is likely ancestral to all downy mildews and many *Phytophthora* species. The genome of *P. effusa* was resolved as being larger and more repeat dense than previously reported. Several paralogous gene families were shown to have been collapsed, including genes encoding crinklers. Some effector genes in *P. effusa* were organized as clusters of high identity genes; clusters of genes encoding crinklers were smaller, on larger chromosomes, clusters of genes encoding RXLR effectors were larger on smaller chromosomes. High-quality, T2T genome assemblies will enable definition of the complete gene space, including resolving paralogous effector encoding genes, and investigate genome evolution from the ancestral architecture for downy mildews and *Phytophthora* species.

Investigating the role of extrachromosomal circular DNAs in fungal plant pathogen evolution

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One of the ways genomes respond to stress is by shedding extrachromosomal circular DNAs (eccDNAs). EccDNAs can contain genes, dramatically increase their copy number, and reinsert into the genome, generating structural variation. However, their role in the genome evolution of filamentous fungi and plant pathogens, has remained virtually unexplored until now. *Magnaporthe oryzae*, the devastating rice and wheat blast pathogen, is easily culturable and has been extensively studied making it an ideal system for this research. We isolated eccDNAs from cultures of *M. oryzae* and sequenced them using both short and long read sequencing. We first designed a bioinformatics pipeline to process our raw sequencing data into eccDNA-forming regions for further analysis. We have since shown that these regions contain many genes, including effectors, and that a specific subset of genes tend to be overrepresented on circular DNAs in *M. oryzae*. We have also found that eccDNAs were significantly enriched in TE-rich and gene-sparse regions of the genome. Our analysis has focused on understanding why specific gene subsets are overrepresented, what characteristics shape the circularome of *M. oryzae* and how these characteristics might differ from other previously studied organisms. Finally, we are also exploring the role of eccDNAs in facilitating structural variation in the *M. oryzae* genome and how eccDNAs could contribute to overcoming host defenses.

The TriMV IRES: Insights into the conserved strategies used by plant viruses in translation

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One common cellular response to stress is the global turn-off of protein synthesis. Many RNA viruses utilize an internal ribosomal entry site (IRES) element to ensure that their translation remains active, overcoming one of the first layers of the defense response. Here, we dissected the IRES activity of Triticum mosaic virus (TriMV, Potyviridae), a recently emerged pathogen of wheat which features characteristics commonly found in animal viruses. The TriMV IRES does not rely on the canonical translation initiation factors often targeted during translation shut-off and features a *cis*-acting polypyrimidine (YX)-AUG motif needed for designation of the 13th AUG located 739 nts downstream of the 5' end, as the preferred initiation site. We propose that TriMV IRES uses this CU-rich track to recruit the 40S ribosomal subunit to the AUG by direct base pairing. Two additional flanking CU-rich segments were identified to likely increase the local concentration of ribosomes to the 3' end of the IRES. SHAPE analysis confirmed the structural availability of these CU-rich regions for potential base pairing. Using ancestral sequence reconstruction, our goal is to infer the potential evolution of the plant-and animal-hosted IRESes. These findings expand our understanding of the core requirements and evolution of IRES elements, which could aid our efforts of finding viral-specific targets for antiviral control strategies.

alfaNET: A database of host-pathogen protein-protein interactions and protein features of *Pseudomonas syringae* causing bacterial stem blight in alfalfa

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Alfalfa has emerged as the most important forage crop, owing to its wide adaptation and high biomass production worldwide. Since the last decade, the emergence of the bacterial stem blight (caused by *Pseudomonas syringae* pv. *syringae* ALF3) in alfalfa has caused around 50% yield losses in the United States. Studies are being conducted to decipher the role of key genes and pathways regulating the disease but due to sparse knowledge about the infection mechanism of *Pseudomonas*, the development of the resistant cultivars is hampered. The database, alfaNET, is an attempt in this direction to assist researchers by providing comprehensive *Pseudomonas* proteome annotation, and host-pathogen interactome tool, which predicts the interactions between host and pathogen. alfaNET is a user-friendly, efficient tool, and includes other features such as subcellular localization prediction, gene ontology (GO) annotations, network visualization and effector protein prediction. The users can also browse and search the database using particular keywords or the proteins with a specific length. Additionally, the BLAST search tool enables the user to perform homology sequence search against the alfalfa and *Pseudomonas* proteomes. With the help of these attributes, alfaNET will be a beneficial resource to the research community engaged in implementing molecular strategies to mitigate the disease. alfaNET is freely available for public use at <http://bioinfo.usu.edu/alfaNET/>.

Acibenzolar-S-methyl controls bacterial blight of Brassicaceae systemically

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Bacterial blight disease of Brassicaceae caused by *Pseudomonas cannabina* pv. *alisalensis* (*Pcal*) is an economically important disease worldwide. Management of bacterial blight based on copper fungicides and antibiotics resulted in the occurrence of a *Pcal* resistant strain.

Therefore, there is an increasing demand for new sustainable disease control strategies. Acibenzolar-S-methyl (ASM), a synthetic analog of salicylic acid (SA), has been used to protect crops from disease by activating plant defense. However, the mechanisms by which ASM contributes to controlling bacterial blight remains unclear. We demonstrated that cabbage inoculated with *Pcal* showed reduced symptoms and bacterial populations 2 h after ASM soil drench treatment. We further investigate the ASM induced systemic protective effect on cabbage and Japanese radish by ASM foliar treatment. ASM suppressed *Pcal* disease on ASM-treated leaves and untreated leaves in both plants, indicating ASM activates systemic plant defense. Since ASM showed protective effects within 2 h, we next examined stomatal-based defense after *Pcal* inoculation with or without ASM. Cabbage and Japanese radish leaves exhibited stomatal closure after ASM treatment. These results indicate that ASM activated stomatal-based defense. Furthermore, ASM-induced stomatal closure was observed both locally and systemically. Taken together, ASM protects cabbage and Japanese radish from *Pcal* by activating stomatal-based defense systemically.

Allelic variants of the NLR protein Rpi-chc1 differentially recognize members of the *Phytophthora infestans* PexRD12/31 effector superfamily through the leucine-rich repeat domain

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Phytophthora infestans is a pathogenic oomycete that causes the infamous potato late blight disease. Resistance (*R*) genes from diverse *Solanum* species encode intracellular receptors that trigger effective defense responses upon the recognition of cognate RXLR avirulence (*Avr*) effectors. In this study, we cloned sixteen allelic variants of the *Rpi-chc1* gene from *Solanum chacoense* and other *Solanum* species, and identified the cognate *P. infestans* RXLR effectors. These tools were used to study effector recognition and co-evolution. Functional and non-functional alleles of *Rpi-chc1* encode Coiled-Coil Nucleotide-Binding Leucine-Rich-Repeat (CNL) proteins. These alleles have distinct patterns of RXLR effector recognition. While Rpi-chc1.1 recognized multiple PexRD12 (*Avrchc1.1*) proteins, Rpi-chc1.2 recognized multiple PexRD31 (*Avrchc1.2*) proteins, both belonging to the PexRD12/31 effector superfamily. Domain swaps between Rpi-chc1.1 and Rpi-chc1.2 revealed that overlapping subdomains in the LRR were responsible for the difference in effector recognition. This study showed that Rpi-chc1.1 and Rpi-chc1.2, evolved to recognize distinct members of the same PexRD12/31 effector family via the LRR domain. The biased distribution of polymorphisms suggests that exchange of LRRs during host-pathogen co-evolution can lead to novel recognition specificities. These insights will help future strategies to breed for durable resistant varieties.

Structure-function analysis reveals motifs necessary for TuMV VPg-eIF(iso)4E interaction are dispensable for CBSV VPg interaction with cassava nCBP2

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Cassava is a crucial food security crop in sub-Saharan Africa. There, a key constraint on production is cassava brown streak disease (CBSD), caused by independent or simultaneous infection with two species of +ssRNA viruses of the family *Potyviridae*, genus *Ipomovirus*: *Cassava brown streak virus* (CBSV) and *Ugandan cassava brown streak virus* (UCBSV). Diseases caused by the family *Potyviridae* generally require interaction of viral genome-linked protein (VPg) and host eukaryotic initiation translation factor 4E (eIF4E) isoforms. Cassava encodes five eIF4E-family members: eIF4E, eIF(iso)4E-1, eIF(iso)4E-2, nCBP-1, and nCBP-2. We previously found that *ncbp-1/ncbp-2* double mutants exhibit reduced CBSD symptoms and storage root viral titer. Interestingly, CBSV VPg associated with all eIF4E-family members by co-immunoprecipitation. Here we demonstrate through pull-down assays that CBSV VPg directly interacts with all cassava eIF4E-family proteins. We also find that certain amino acids critical for *Arabidopsis* eIF(iso)4E–TuMV VPg interaction are dispensable in CBSV VPg interaction with cassava nCBP-2. Furthermore, threading of CBSV and TuMV VPg onto the NMR structure of PVY VPg reveals that the “ $\alpha 1$ - $\alpha 2$ loop” 4E–interaction domain of PVY and TuMV VPg are similar, but the length of this structure is shortened in CBSV VPg. Taken together, these data suggest that VPg of CBSV, compared to those of other potyvirids, has evolved different means of interacting with host susceptibility factors.

Differential expression of effector-encoding genes in two clades of the cucurbit downy mildew pathogen *Pseudoperonospora cubensis*

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Downy mildew caused by the obligate oomycete pathogen *Pseudoperonospora cubensis* is one of the most devastating diseases of cucurbit crops. Recent studies have identified two phylogenetically distinct clades of *P. cubensis*, Clade 1 and Clade 2, that show differences in host preferences. Preliminary data suggests that the introduction of Clade 2 to the United States was responsible for the downy mildew epidemic in 2004. We sequenced one isolate each of Clade 1 and Clade 2 with an aim to identify effectors in both clades. Secretomes of both clades were predicted using SignalP and TMHMM. Putative RXLR effector candidates were identified using the R package effectR and a web-based Galaxy toolshed specifically trained for oomycete effectors. There were ~1,000 predicted secreted proteins and ~200 RXLR effectors in the two clades. An RNA-seq time-course experiment performed with samples collected at 3-, 4-, 5- and 6-days post infection showed differential expression of some effector-encoding genes between the two clades. Some of the selected clade-specific and clade-nonspecific genes showed differential RT-qPCR expression patterns. Expression patterns of some of these genes were validated in other Clade 1 and Clade 2 isolates. Future downstream functional analysis of these putative effectors is being done to select candidates for effector-assisted breeding to identify new sources of resistance to downy mildew in cucurbits.

Type III secretion system effectors and host specificity in *Pseudomonas savastanoi* pathovars of woody hosts

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The species *Pseudomonas savastanoi*, a member of the *Pseudomonas syringae* complex, includes five pathovars causing knots or excrescences in woody hosts: *P. savastanoi* pv. *savastanoi*, pv. *fraxini*, pv. *nerii*, pv. *retacarpa* (Psr), and pv. *mandevillae*, comprising isolates from olive, ash,

oleander, broom and dipladenia plants, respectively. Comparative genomic analysis of all available *P. savastanoi* genomes included in these five pathovars defined a total of 45 type III secretion system effector (T3E) genes, including 24 core genes, four genes exclusive of Psr and several genes encoding pathovar-specific truncations. Noticeably, hierarchical clustering based on the presence, absence and truncations of T3E genes, correlated with the core genome phylogeny of these strains, indicating that the five pathovars contain characteristic sets of T3Es, which are thus likely contributing to define their pathogenicity profile. To unravel the role of T3Es in the host specificity of *P. savastanoi* pathovars we followed two different approaches: i) construction of knock out mutants affected in T3E genes exclusively encoded by specific pathovars and, ii) heterologous expression of T3E genes in strains belonging to pathovars lacking these T3Es or encoding specific truncations. Virulence assays of these strains in their natural hosts and in hosts infected by other *P. savastanoi* pathovars, allowed us to identify several T3Es with a role in the host specificity of this bacterial pathogen of woody hosts.

Multi-layered characterization of the barley interactome links MLA immune receptor signaling with defense gene expression

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Obligate fungal pathogens (e.g., mildews and rusts) are a major threat to cereal grain production worldwide. The barley MLA immune receptor and its orthologs confer recognition specificity to many important cereal diseases, including powdery mildew, stem and stripe rust, Victoria blight, and rice blast. To characterize the signaling networks associated with MLA-based immunity, we incorporated an interactome-based protein interactome with an infection-time-course transcriptome and eQTL information. Integration of interactome and eQTL data enabled us to infer disease modules associated with powdery mildew penetration and haustorial development, revealing both core and unique responses at each developmental stage. Then, using interactome and transcriptome data, we assembled resistant (R) and susceptible (S) subnetworks by selecting significant co-expressed interactions under each defense phenotype. Proteins in the differentially co-expressed (R-S) subnetwork are essential to barley immunity, connect and facilitate the flow of signaling pathways, and are linked to *Mla* through eQTL associations. Lastly, next-generation, yeast-two-hybrid assays identified fourteen novel MLA interactors, which were overlaid onto the interactome, predicting the immune-receptor cellular localization, and signaling response over time. Our findings highlight master components of the NLR signaling cascade, linking genomic, transcriptomic, and physical interactions during the MLA-based immune response.

Genome-based prediction of the type III secretion system effector repertoire of Chilean strains of *Pseudomonas syringae* pv. *tomato* compared with DC3000 and T1-like strains

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In recent years, severe symptoms of bacterial speck disease caused by *Pseudomonas syringae* pv. *tomato* (*Pto*), have been observed increasingly in Chilean tomato fields. Type III secreted effector (T3SE) proteins are essential virulence determinants of *P. syringae*. Additionally, the effector repertoires of the *Pto* DC3000 and T1 reference strains have been reported to exhibit marked differences, even though they cause the same disease in tomato, differing in the host range. Despite being a frequent tomato disease in Chile, the effector repertoires of Chilean *Pto* strains are unknown. The aims of this work were to identify the virulence determinants present in the genomes of Chilean *Pto* strains, specifically the effector repertoire, and compare them with *Pto* strains DC3000, T1 and B13-200. The genomes of three *Pto* strains were completely sequenced. A prediction of T3SEs was carried out, revealing that the three strains had the same effector repertoire, consisting of 35 T3SEs. These 35 T3SEs are shared with strain B13-200 that presented one additional T3SE described in DC3000; 34 T3SEs are shared with strain T1, and 28 T3SEs with strain DC3000. The location of genes, the presence of gene clusters and genomic islands was also analyzed. The results of this study indicate that the Chilean *Pto* strains analyzed correspond to T1-like strains. The additional T3SE observed in Chilean strains and B13-200 needs to be studied to verify their effects on the virulence in different plant hosts.

Expression analysis of the Xa21 resistance gene in sugarcane in response to hormone treatments

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Xa21, a resistance gene identified and characterized firstly in rice, is a member of a multigene family, which confers resistance to several strains of the bacterial pathogen from *Xanthomonas* sp. Leaf scald caused by *Xanthomonas albilineans* is a primary and significant bacterial disease of sugarcane, but the molecular mechanisms of resistance to leaf scald are still unclear. The present study identified orthologous Xa21 in many *Poaceae* species, as well as ten candidate genes of the Xa21 family in sugarcane (ShXa21-1 to ShXa21-10) arranged in tandem duplication. We identified the putative *cis*-acting elements in the promoters of ShXa21 genes using the online tool PlantCARE. The results reveal that ShXa21 genes contained many hormone-responsive *cis*-elements in their promoter, suggesting that hormones might control their expression. Therefore, we investigated the effects of phytohormone mainly related to plant defenses, such as jasmonate, salicylic acid and the ethylene-releasing compound ethephon. Sugarcane plantlets were exposed to those hormones for 3, 24, and 48 h. Our analysis indicated that the expression of ShXa21-5 and ShXa21-10 varied after 3 h of treatment with ethylene and jasmonate, suggesting that these genes could be activated rapidly by these hormones. This study points to a potential contribution of the ethylene and jasmonate signaling pathways to the Xa21-mediated resistance in sugarcane.

The genome of *Ciborinia camelliae*, a pathogen specific to camellia flowers

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Ciborinia camelliae Kohn is the causal agent of camellia flower blight. The pathogen infects only flowers of camellias, causing serious damage to the plant and economic damages to growers. European diversity of the strains was analyzed by multilocus sequencing targeting GAPDH, EF1a, B-TUB2, and ITS regions. Strain diversity showed that some isolates obtained in Europe may originate from other regions where recombination may have occurred. To better decipher the genetic determinants of virulence in this species, the full genome sequence of an Italian strain of *C. camelliae* was obtained with a hybrid approach combining Illumina Hiseq paired reads and MinIon Nanopore long reads sequencing. We obtained a genome with a total length of 48.01 Mb and 97.5% completeness according to BUSCO analysis. The genome was assembled into 55 contigs with a contig N₅₀ of 2.01 Mb. Our work extends the knowledge on *C. camelliae* diversity and contributes to improving the knowledge on genomes of an important family of plant pathogens: Sclerotiniaceae.

Exposing the catalog of NLR genes in cultivated hexaploid sweetpotato

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Cultivated for its nutritious roots, sweetpotato (*Ipomoea batatas*) is an important staple crop in the tropics, especially in sub-Saharan Africa, Central America, and New Guinea. Sweetpotatoes are highly susceptible to diverse pathogens in the field and postharvest. Breeding sweetpotatoes for resistance remains puzzling due to limited knowledge on the genetic basis of resistance and the hexaploid nature of the crop. Plant intracellular immune receptors known as nucleotide-binding domain leucine-rich repeat receptors (NLR) represent a key component of the plant immune system by mediating plant immune response to pathogen threats. Here, we aimed to catalog NLR diversity in historical (Apache) and contemporary (Covington) sweetpotato cultivars. We used resistance gene enrichment sequencing (RenSeq), a genome reduction approach, to capture and sequence full NLRs. A custom designed NLR bait-library of 2,034 targets allowed us to enrich NLR genes with 88% target capture rate. Using PacBio sequencing after RenSeq on genomic DNA, we identified 649 and 833 complete NLRs for Covington and Apache, respectively. To uncover the diversity of NLRs in sweetpotato, we employed a curated database of cloned and functionally characterized NLRs (RefPlantNLR) to assign sequenced sweetpotato NLRs to functional phylogenetic clades. Our study provides a catalog of NLR genes that will accelerate breeding and improve our understanding of evolutionary dynamics of NLRs in sweetpotato.

Effector redundancy and interference in *Psa*

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Pseudomonas syringae pv. *actinidiae* biovar 3 (*Psa3*) is a destructive pathogen of kiwifruit plants (*Actinidia* spp.). Our previous work using targeted gene knockouts identified several effectors secreted via the type III secretion system (T3SS) critical for the pathogenicity of this pathogen. Interestingly, the largely non-pathogenic, closely related strain *P. syringae* pv. *actinidifoliorum* (*Pfm*) ICMP 18803 carries the very same disease-critical effectors as the canker-causing *Psa3* strain ICMP 18884. We used *Pfm*, and targeted multiple-effector knockouts in *Psa3*, to assess effector contribution and discovered layered interference and redundancy present in the *Psa3* effector repertoire. Effectors *hopZ5*, *hopH1*, *avrPto5*, *hopBB1* (two copies), and *avrRpm1* show a redundant contribution to pathogenicity. Additionally, *HopZ5*, *HopZ3*, and *AvrRpm1* show a redundant suppression of HopF2-triggered immunity. Understanding the convoluted modes of *Psa3* effector function offers glimpses at opportunities for developing a layered, durable effector-triggered resistance strategy.

Understanding the evolutionary origin of novel receptor functions using INR, a PRR for detecting herbivory specific to the legume tribe Phaseoleae

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Pattern recognition receptors (PRRs) detect diverse pathogen and pest-associated molecular patterns (PAMPs), but PRR recognition specificity is not widely explored beyond individual species. To establish a model for the emergence of lineage-specific PRR functions, we analyzed the LRR-RLP Inceptin Receptor (INR), which detects conserved inceptin peptides found in caterpillar oral secretions. Consistent with the restricted bioactivity of inceptin, INR is specific to the genomes of select legume species in the Phaseoleae. To understand the ancestral emergence of INR, we used a combined comparative genomic and functional approach. We constructed genome assemblies of early-diverging legume species which were differentially responsive to inceptin. Oxford Nanopore-based long reads yielded scaffolds spanning the complex INR locus, revealing 0-7 tandem receptor duplicates per species. Heterologous assays in tobacco defined a clade of inceptin-responsive orthologs with branch- and site-level signatures of positive selection relative to non-functional homologs at the same locus. We present an evolutionary model entailing 1) receptor gene insertion at the ancestral locus, 2) paralog diversification associated with gain of INR function, and 3) stabilization of a two-gene locus providing extant INR functions in *Vigna* and *Phaseolus* crop species. Understanding the evolution of INR provides a framework for predicting genes with novel PRR functions in specific plant lineages.

HCPPro suppression of callose deposition as a conserved potyviral function in pathogenicity

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The Helper Component Protease (HCPPro) protein, encoded by viruses in the family *Potyviridae*, functions in the infection cycle and as a pathogenicity determinant. In fact, the pathogenicity of Potato virus Y (PVY) on some strain-specific resistant potato cultivars is defined by residues within the HCPPro C-terminal region, with a specific signature resulting in a host hypersensitive response for the common PVY^O strain and the evasion of a defense response for the necrotic strain PVY^{N:O}. We previously showed that HCPPro also suppresses callose accumulation and that recognition of HCPPro^O by host resistance genes overcomes this function. For instance, in potato cultivars resistant to PVY^O, HCPPro^N

suppresses callose accumulation and HCPro^O does not. Swapping the signature region of HCPro^O with that of HCPro^N restored the ability of HCPro^O to suppress callose accumulation. The single-point mutation of a serine in the HCPro^O signature region with the alanine in the same position in HCPro^N results in callose suppression and no host defense response as observed with HCPro^N, and mutating the alanine in HCPro^N to a serine leads activity observed with HCPro^O. Furthermore, substituting the serine in HCPro^O with phosphomimetic residues retained the wild type phenotype, linking a potential role of phosphorylation in host recognition. We are investigating the conservation of HCPro function in callose suppression within the *Polyviridae* family, implicating a new viral function in pathogenicity.

Association of two co-occurring mutations at the AvrMlp7 avirulence locus in poplar rust during the breakdown of RMLp7 resistance gene

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The deployment of plant varieties carrying resistance (*R*) genes exerts strong selection pressures towards pathogen populations. Several studies reported the rapid evolution of avirulence (*Avr*) genes to escape *R*-mediated plant immunity and identified a variety of mechanisms leading to virulence. The poplar rust fungus *Melampsora larici-populina* is the most damaging pathogen of poplars. A major adaptive event occurred in 1994 with the breakdown of *RMLp7* resistance gene in poplar in Europe. Population genomics studies identified a locus in the genome of *M. larici-populina*, which likely corresponds to the *AvrMlp7* candidate avirulence gene. We used a population genetics approach combined with dedicated qPCR assays on a comprehensive set of 281 isolates, covering 27 years (encompassing the resistance breakdown event), to validate the candidate locus and to assess its polymorphism. We found two mechanisms, a point mutation and a deletion, that allowed the pathogen to escape *RMLp7*-mediated resistance. Six diploid genotypes were thus characterized at the candidate locus (three avirulent and three virulent). In addition, a temporal analysis revealed that the two virulence alleles pre-existed (harbored as avirulent heterozygous genotypes) since the early samplings and were found in association (as virulent genotypes) at the time of the resistance breakdown. Our study documented that, in a diploid pathogen, combining virulence determinisms is adaptive.

***Xanthomonas euvesicatoria* gene Xe4429 encodes a protein functions as an antitoxin, a transcription repressor, and an enhancer of XAvrRxol secretion**

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Xanthomonas euvesicatoria is the causal agent of bacterial spot disease that threatens pepper and tomato production around the globe. *X. euvesicatoria* gene *Xe4428* encodes a type III effector (T3E) that shares 89.67% amino acid identity with *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) T3E *AvrRxol*. Deletion of *Xe4428* in the genome of *X. euvesicatoria* (strain Xcv85-10) compromised its virulence to infect pepper and *Nicotiana benthamiana* plants. Transient co-expression of *Xe4428* and *Rxo1* on pepper and *N. benthamiana* plant leaves results in a strong hypersensitive reaction. Thus, *Xe4428*, renamed as *XeAvrRxol*, is a bona fide orthologue of *XocAvrRxol* that possesses both virulence and avirulence functions. Expression of *XeAvrRxol* is toxic in *E. coli* and *X. euvesicatoria* cells. Another *X. euvesicatoria* gene *Xe4429*, encodes a putative chaperone of *XeAvrRxol*, which can interact with *XeAvrRxol* to suppress its toxicity in *X. euvesicatoria* and *E. coli* bacterial cells. *Xe4429* also binds to the promoter region of *XeavrRxol* and represses its transcription in *X. euvesicatoria* bacterial cells. In addition, expression of *Xe4429* can enhance the secretion and translocation of *XeAvrRxol* into plant cells. Therefore, *Xe4429* functions as an antitoxin, a transcription repressor, and a type III chaperone that is capable of enhancing the secretion and translocation of *XeAvrRxol* during pathogenesis.

Comparative genomics reveals lineage-specific effector genes in *Alternaria solani* and evidence of horizontal gene transfer

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Comparative genomic analysis of the genus *Alternaria* identified two lineage-specific effector genes that are only present in the potato and tomato early blight pathogens *A. solani* and *A. tomatophila*. The lineage-specific effector genes, *AsCEP19* and *AsCEP20*, constitute a “head-to-head” gene pair located near an AT-rich region in the *A. solani* genome. Phylogenetic inference confirmed that the effector genes were horizontally transferred from *Corynespora cassicola* strain Philippines. The qRT-PCR analysis showed that the gene pair is tightly coexpressed and only upregulated at advanced stages of *A. solani* infection in potato plants. Transient expression of the effector genes in *Nicotiana benthamiana* showed that they are both able to suppress PAMPs-triggered ROS burst, indicating that *AsCEP19* and *AsCEP20* can attenuate the PTI responses. Transient expression of *AsCEP19* did not cause phenotypic alterations in *N. benthamiana*, whereas *AsCEP20* is able to induces plant cell death depended on the presence of its signal peptide. Subcellular localization analysis revealed that both *AsCEP19* and *AsCEP20* are localized in the chloroplasts, and *AsCEP19* is also able to localized to the plasma membrane. Our results suggest that *AsCEP19* and *AsCEP20* play important roles in the pathogenicity of early blight pathogens, and probably involved in the adaption of early blight lineage to solanaceous crops.

Is fidelity a strength? Aligning evolution to pathogen host range. A case study of RxLR effectors in *Phytophthora* spp.

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The genus *Phytophthora* includes species of diverse hosts that, in part, sustain success by engineering a constant evolution of their RxLR effector repertoire. This attribute is common among *Phytophthora* spp. but its role to delimit host range is unclear. Here, we exploit in silico platforms to examine the distribution and functional features of RxLR effector repertoires in five *Phytophthora* spp. towards their potential role to delimit pathogen host range. To this end, comparative analyses in distribution of species specific clusters of orthologous groups reviewed an expansion of unique putative RxLR effectors in narrow, but not broad hosts *Phytophthora* spp. The species specific RxLR effectors included 52 *P. infestans*, 30 *P. sojae*, 14 *P. palmivora*, 6 *P. cactorum* and 3 *P. parasitica*. We defined a set of 100 putative ‘core’ RxLR effectors from seven clusters of orthologous groups with evolutionary conservation across all examined *Phytophthora* spp. GO analyses of the species specific and core RxLR effectors reviewed unique attributes in pathogenesis that may influence host range. Collectively, our results implicate a variable ability by *Phytophthora* spp. to align a common attribute in evolution of their RxLR effector repertoire to delimit host range. Indeed, to maintain fidelity to

a host might be a strength and experimental validation of the unique RxLR effector sets identified, we hope, could shed light on why some *Phytophthora* spp. need more RxLR effectors than others.

Tandem protein kinases emerge as new regulators of plant immunity

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Plants actively resist pathogens via a complex immune system comprised of surface-localized and intracellular receptors. To date, the majority of cloned resistance genes encode intracellular nucleotide-binding leucine-rich repeat (NLR) proteins. Recent studies have revealed that the Tandem Kinase Proteins (TKPs) are an important family of intracellular proteins involved in plant immune responses. The *Yr15* (*WTK1*) TKP gene protects wheat against stripe (yellow) rust fungal infection. EMS mutagenesis and transgenic approaches enabled validation of WTK1 function and confirmed that both kinase-like domains are essential to provide resistance. Its introgression into multiple wheat genetic backgrounds was shown to result in variable phenotypic responses, which suggests that *WTK1*-mediated resistance is part of a complex immune response network. In addition to WTK1, several genes coding for TKPs and protecting against devastating fungal diseases have been isolated: barley *Rpg1* and wheat *WTK2* (*Sr60*), *WTK3* (*Pm24*), and *WTK4*. Moreover, TKPs are widespread and diverse across the Plant kingdom, are of polyphyletic origin through duplication or gene fusion and represent an example of structural convergent evolution. A better understanding of the evolution of TKPs and their molecular function is important to support future exploitations including gene manipulation and synthetic engineering of novel resistance genes.

A 20-kb lineage-specific genomic region tames virulence in pathogenic amphidiploid *Verticillium longisporum*

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Ascomycetous fungi of the genus *Verticillium* are worldwide distributed vascular plant pathogens. The amphidiploid *Verticillium longisporum* evolved from at least three separate hybridizations of haploid parental *Verticillium* strains. We compared the genomes and virulence of two *V. longisporum* isolates from rapeseed fields in the same area of Germany to understand the basis of their different ability to cause disease symptoms in the plant host *Brassica napus*. Although V132 and V143 similarly penetrate plant roots, proliferation of asymptomatic V132 in planta is lower than of virulent V143, which induces strong disease symptoms. The highly conserved V143 and V132 genomes include less than 1% unique genes, and the karyotypes of 15 or 16 chromosomes display changed genetic synteny due to substantial genomic reshuffling. A 20 kb V143 lineage-specific (LS) region originating from the *V. dahliae*-related ancestor is specific for symptomatic V143 and encodes seven genes, including two putative transcription factors. Plant pathogenicity experiments revealed that partial or complete deletion of the LS region in V143 does not reduce virulence but leads to the induction of more severe disease symptoms in rapeseed. This suggests that the LS insertion in the genome tames symptomatic *V. longisporum* V143 from being even more virulent and restricts the damage on the host plant. Regions required for pathogenicity attenuation help to control fungal growth, therefore supporting disease management.

The PXO99a type III TAL effector Tal9b targets the OsFLS3 gene to promote virulence in rice

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The rice pathogen *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) employs Transcription Activator-Like Effectors (TALEs) secreted through the type 3 secretion system (T3SS) to enhance expression of host susceptibility factors. TALEs induce the expression of the cognate target genes in the host by binding to the effector-binding element (EBE) in the promoter. A meta-analysis of multiple gene expression data indicated higher expression levels of rice flavonol synthase (*OsFLS3*) upon *Xoo* treatment. In silico analysis predicted the presence of an EBE in the promoter of *OsFLS3*, and the *Xoo* PXO99^A Tal9b to be the putative TALE that targets this EBE. Treatment of rice plants with *Xoo* mutants defective in the T3SS and Tal9b showed poor and reduced induction of *OsFLS3* transcripts than the wild type, respectively, indicating the T3SS-dependent induction of *OsFLS3* by *Xoo* and that Tal9b is likely the TALE that is responsible for induction. Tal9b is essential for complete virulence of *Xoo* as the Tal 9b mutant caused reduced lesions in rice leaves. *AtDMR6*, the homologue of *OsFLS3* in *Arabidopsis thaliana*, is a susceptibility factor for *Pseudomonas syringae* pv. *tomato* (*Pst*). Ectopic expression of *OsFLS3* in *atdmr6* plants restored susceptibility to *Pst* infection indicating a functional homology between *OsFLS3* and *AtDMR6*. Taken together, this study identifies a putative rice susceptibility gene targeted by *Xoo* and expands the functional diversity of the *Xoo* TALE targets in rice.

Proteome analysis of phloem tissue from two rootstocks with opposite degrees of susceptibility to *Citrus tristeza virus*

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Citrus plants can be seriously affected by the *Citrus tristeza virus* (CTV), a ssRNA(+) phloem limited *Closterovirus*. Rootstocks *Citrus macrophylla* (CM) and *C. aurantium* (CA) have differing susceptibility to CTV, with the latter species being less susceptible. The susceptibility has been correlated with high viral titers in the phloem tissue, with CA being a genotype that accumulates a very low viral titer compared to CM. To investigate the molecular response of CA and CM to CTV, the phloem sap proteome of plants of each species infected with the isolate T36 and uninfected controls were analyzed by nanoLC-MS/MS. A total of 5413 proteins were quantified in CM with a group of 39 differentially expressed (DE) proteins ($p < 0.05$, fold change ≥ 2 or ≤ 0.4) between infected and control plants, while 59 out of a total of 5681 quantified proteins were DE in infected versus control CA plants. Significant differences were found between the infected CM and CA proteome. Proteins involved in flavonoid biosynthesis and nitrogen metabolism were significantly modified in CM, while proteins involved in citrate cycle and pyruvate were significantly modified in CA. In summary, this approach highlighted distinct mechanisms in each rootstock that may explain the

different susceptibility to CTV infection of these two genotypes. Research supported by FCT, Portugal (PTDC/BAA-AGR/30957/2017) and FEEI (CRESC 2020 ALG-01-0145-FEDER-30957). LA funded by Norma Transitória_FCT-DL57/2016/CP1361/project CT0011 (www.sciencedirect.com/science/article/pii/S0300908420300365?via%3Dihub#gs15).

A pair of effectors encoded on a conditionally dispensable chromosome of *Fusarium oxysporum* f. sp. *conglutinans* suppress Arabidopsis-specific immunity

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Many plant pathogenic fungi contain conditionally dispensable (CD) chromosomes that are associated with virulence, but not growth in vitro. Virulence-associated CD chromosomes carry genes encoding effectors and/or host-specific toxin biosynthesis enzymes that may contribute significantly to determining host specificity. *Fusarium oxysporum* causes devastating diseases of more than 100 plant species. Among a large number of host-specific forms, *F. oxysporum* f. sp. *conglutinans* (*Focn*) can infect Brassicaceae plants including Arabidopsis (*Arabidopsis thaliana*) and cabbage. Here we show that *Focn* has multiple CD chromosomes. We identified specific CD chromosomes that are required for virulence on Arabidopsis, cabbage, or both, and describe a pair of effectors encoded on one of the CD chromosomes that is required for suppression of Arabidopsis-specific phytoalexin-based immunity. The effector pair is highly conserved in *F. oxysporum* isolates capable of infecting Arabidopsis, but not of other plants. This study provides insight into how host specificity of *F. oxysporum* may be determined by a pair of effector genes on a transmissible CD chromosome.

Standing genetic variation of the AvrPm17 gene in powdery mildew limits the effectiveness of the introgressed rye Pm17 resistance gene in wheat

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Introgressions of chromosomal segments from related species into wheat are important sources of resistance. The durability and effectiveness of introgressed resistance genes upon agricultural deployment is highly variable—a phenomenon that remains poorly understood as the corresponding fungal avirulence genes are largely unknown. Until its breakdown, the *Pm17* resistance gene introgressed from rye to wheat provided broad resistance against powdery mildew (*Blumeria graminis*). Here, we used QTL mapping to identify the corresponding wheat mildew avirulence effector *AvrPm17* encoded by two paralogous genes. Haplovariant mining in wheat mildew and related sub-lineages identified several ancient virulent *AvrPm17* variants that were present in wheat powdery mildew before the *Pm17* introgression, thereby paving the way for the rapid resistance breakdown of *Pm17*. QTL mapping in mildew identified a second genetic component likely corresponding to an additional resistance gene present on the 1AL.1RS translocation carrying *Pm17*. We conclude that the initial effectiveness of 1AL.1RS was based on simultaneous introgression of two genetically linked resistance genes. Our findings demonstrate the relevance of pathogen-based genetic approaches to study complex resistance loci in wheat. We propose that identification and monitoring of avirulence gene diversity in pathogen populations becomes an important part of introgression breeding to achieve durable resistance in wheat.

Bioinformatics pipeline for MAX family effectors prediction

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Fungi effectors presents hyper-diversity making challenging to probe good criteria to identify structural related function. We overcome the methodological and conceptual barrier by building on our recent discovery of an important, structurally conserved, but sequence-diverse family of effectors called MAX (*Magnaporthe* AvrS and ToxB). The possibility to identify in silico a reasonably-sized class of effectors allows to tap into the natural variability within *Magnaporthe oryzae* to provide hypotheses to be tested using molecular plant biology. We develop advanced bioinformatics pipelines to massively and accurately identify MAX effectors and model their 3D structure. By integrating in silico structure determination from sequence alone and experimental validation combining NMR and X-ray crystallography, we report in these preliminary work important clues for implementing and further straightening the quality of a prediction pipeline. Having in hands such a tool gives the opportunity to decipher on evolution pathways and the function of the effectors starting from their sequences.