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A casein kinase modulates PARylation in *Arabidopsis* immunity and DNA damage response

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Poly(ADP-ribosylation) (PARylation) is a reversible post-translational modification process catalyzed by poly(ADP-ribose) polymerases (PARPs). PARPs transfer ADP-ribose moieties from NAD⁺ to the target proteins. Protein PARylation has been implicated in DNA damage and repair, cell cycle, genotoxic stress, circadian rhythms, gene regulation, and immunity. However, it remains elusive how PARP activities are regulated and link to specific biological pathways. We show here that casein kinase CK2 α , a ubiquitous serine/threonine kinase conserved in all eukaryotes, interacts with and directly phosphorylates PARP1 and PARP2 in *Arabidopsis*. Furthermore, microbe-associated molecular pattern (MAMP) and genotoxic stress promote the binding of CK2 α 1 with PARP2. The *ck2 α* mutants show increased susceptibility to bacterial pathogen infections, decreased PARylation under genotoxic stress, and increased sensitivity of pathogen-triggered DNA damage. Taken together, our research reveals that CK2 α 1 plays a crucial function in *Arabidopsis* immunity and DNA damage response by regulating PARP2 phosphorylation.

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.

Utilizing quantitative proteomics to investigate rapid changes in the plasma membrane proteome during pattern triggered immunity
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In *Arabidopsis thaliana*, loss of Mitogen-Activated Protein Kinase Phosphatase 1 (MKP1), a negative regulator of plant defense, results in plants with enhanced resistance to bacterial infection by *Pseudomonas syringae*. Resistance in *mkp1* mutants is due to decreased accumulation of extracellular bioactive plant metabolites that induce the bacteria's type III secretion system. We hypothesize that this reduction in *mkp1* extracellular metabolites is caused by a rapid decrease in the abundance of plasma membrane (PM) transporters for these phytochemicals. As resistance in *mkp1* resembles wildtype plants pretreated with a PAMP, we also hypothesize that similar changes in the PM proteome occur during PTI responses. We performed a label-free LC-MS/MS analysis of enriched PM fractions from naive *mkp1* plants as well as naive and PAMP-elicited (10 μ M elf18, 1 h) WT plants. Of the 7,337 proteins identified, 12 membrane proteins were decreased in both naive *mkp1* and PAMP-treated WT plants. Immunoblot analyses for one of these proteins confirmed the proteomics results. We also found 24 proteins decreased only in WT after PAMP treatment, indicating that only a subset of the total PAMP-responsive PM changes are dependent on MKP1. These experiments also revealed that proteins, in addition to the pattern recognition receptors, may be rapidly removed from the PM during PTI. This suggests a broader regulatory trafficking process than previously known.

A trimeric CrRLK1L-LLG1 complex regulates NLR protein SUMM2 activation via MEKK2 scaffolding

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Plants have evolved both cell surface-resident pattern recognition receptors and intracellular nucleotide-binding leucine-rich repeat (NLR) proteins to detect infections. It remains enigmatic how PRR- and NLR-mediated signaling is connected in immune response. Disruption of an PRR-activated MAPK cascade consisting of MEKK1-MKK1/2-MPK4 activates NLR SUMM2 via MEKK2, leading to cell death and autoimmunity. To gain insights into the mechanisms underlying SUMM2 activation, we deployed an RNAi-based genetic screen for LETHALITY SUPPRESSOR of *mekk1* (LETUM or LET). A trimeric complex, consisting of a pair of *Catharanthus roseus* receptor-like kinase 1-like (CrRLK1L), LET1/2, and the glycosylphosphatidylinositol (GPI)-anchored protein LLG1, was identified as specific regulators of *mekk1-mkk1/2-mpk4* cell death and acts genetically downstream of MEKK2 in regulating SUMM2 activation. LLG1 functions as a co-receptor of LET1/2 and mediates LET1/2 transport to the plasma membrane. Additionally, LET1/2 complex with both MEKK2 and SUMM2. MEKK2 scaffolds LET1 and SUMM2 for protein stability and association, and counter-regulates the F-box protein CPR1-mediated SUMM2 ubiquitination and degradation; thereby, regulating SUMM2 accumulation and activation. Our study indicates that a trimeric complex, LET1/2-LLG1, senses the perturbation of cellular homeostasis caused by the deficiency in PRR-activated signaling, and activates the NLR SUMM2-mediated autoimmunity via MEKK2 scaffolding.

The homeostasis of oligogalacturonides (OGs) in immunity and development

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The recognition of endogenous molecules acting as “Damage-Associated Molecular Patterns” (DAMPs) is a key feature in the plant immunity. Oligogalacturonides (OGs), cell wall fragments derived from the degradation of the homogalacturonan, are a well-known class of DAMPs. The accumulation of OGs in vivo is favored by the interaction of microbial polygalacturonases (PGs) with plant PG-inhibiting proteins (PGIPs). Transgenic *Arabidopsis* plants expressing an inducible PGIP-PG chimera, named “OG machine” (OGM), have been generated, allowing the OG-release on command in planta. If the release of OGs persists plant growth is inhibited and plants eventually die, reflecting the role of the OGs in the so-called growth-defense “trade-off”. A homeostatic control, which prevents deleterious effects of the over-accumulation of OGs, may rely on the oxidation of OGs, which impairs their signaling activity. Four OG oxidases, named OGOX1-4, that belong to the Berberine Bridge Enzyme-like (BBE-like) super-family have been identified in *Arabidopsis*. In this work the effects of the altered expression of OGOX1 on the defense responses induced by OGs and on resistance to pathogens have been investigated by reverse genetics. Moreover, the spatial features of *OGOX1* expression during development and in response to elicitors and pathogens using promoter::reporter gene fusions is described. Our results show that OGOXs are regulators of the homeostasis of OGs.

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 “ α 1- α 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.

The TIR-NBS-LRR protein CSA1 is required for autoimmune cell death in *Arabidopsis* pattern recognition co-receptor bak1 and bir3 mutants

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The BIR1-associated kinase BAK1/SERK3 is a positive regulator of multiple leucine rich receptor kinase-mediated signaling pathways including pattern triggered immunity (PTI). Absence or overexpression of BAK1 leads to spontaneous cell death formation. BAK1-interacting receptors (BIR) constitutively interact with BAK1, and plants lacking or overexpressing BIR proteins phenocopy the cell death symptoms observed in *bak1* knock outs or overexpressors. In the interactome of BIR3, the TIR-NBS-LRR protein CONSTITUTIVE SHADE-AVOIDANCE 1 (CSA1) was identified by mass spectrometry. CSA1 physically interacts with BIR proteins and can be detected in complexes with BAK1. Direct interaction was shown only for CSA1 with BIR proteins but not BAK1. Double mutant *bak1 bir3* genotypes develop strong dwarfism and cell death symptoms that are dependent on EDS1 and salicylic acid. Loss of CSA1 blocks *bak1* and *bak1 bir3*-mediated cell death formation thus demonstrating that CSA1 is causal for this type of cell death. We propose that CSA1 guards BIR proteins and initiates autoimmune cell death that is observed when BAK1 BIR complexes are impaired. Our findings reveal how cell death in the absence of BAK1 and BIR3 is executed and links BAK1, a common co-receptor of many pattern recognition receptors, to NLR proteins typically implicated in effector-triggered immunity.

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.

Bacterial effectors target plasmodesmata to promote virulence in *Arabidopsis*

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A hallmark of multicellular organisms is their ability to maintain physiological homeostasis by communicating among cells, tissues, and organs. In plants, intercellular communication is largely dependent on plasmodesmata (PD). Upon immune stimulation, plants close PD as part of their immune responses. We show that the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000 deploys an effector protein, HopO1-1, that modulates PD function. HopO1-1 is required for *Pst* DC3000 to spread locally to neighboring tissues during infection. Expression of HopO1-1 in *Arabidopsis* increases the distance of PD-dependent molecular flux between neighboring plant cells. HopO1-1 physically interacts with and destabilizes the plant PD-located protein PDL7 and possibly PDL5. Both PDLs are involved in bacterial immunity. The findings reveal that a pathogenic bacterium utilizes an effector to manipulate PD-mediated host intercellular communication for maximizing the spread of bacterial infection. In addition, we show that at least 16 *Pst* DC3000 effectors have the capacity to move from transformed cells to the surrounding plant cells in a PD-dependent manner. Expression of PDL5 and PDL7 as well as flg22 (a 22-amino acid peptide of bacterial flagellin) treatment lead to PD closure and inhibit the PD-dependent movement of a bacterial effector HopAF1. Further research should reveal the function of mobile effectors in modulating non-cell-autonomous immune responses in plants.

A prenylated Rab acceptor modulates NLR microdomain nesting and rice blast resistance

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Nucleotide-binding site leucine-rich repeat (NLR) receptors, with different subcellular localizations, mediate pathogen effector-triggered immunity (ETI) in plants. The rice NLR PigmR confers broad-spectrum resistance to the blast fungus *Magnaporthe oryzae* without yield penalty. However, how PigmR traffic and plasma membrane-localization are regulated during immune responses remains unknown. Here, we report that a monocot-specific PRA (Prenylated Rab Acceptor) protein, PIBP4 (PigmR-INTERACTING and BLAST RESISTANCE PROTEIN 4), specifically interacts with PigmR and the active form of the Rab GTPase, OsRab5a, thereby loading PigmR on trafficking vesicles targeting and tethering to microdomains in the plasma membrane. PigmR recruits and activates OsRac1, a small GTPase that functions in reactive oxygen

species signaling and the hypersensitive response, in microdomains to ensure ETI during pathogen infection. Thus, our study discovered a PRA-Rab signaling hub of NLR-mediated ETI and a previously unknown mechanism that links the membrane trafficking machinery with NLR-mediated immunity in plants.

Control of FLS2-triggered immunity by GOLVEN phyto cytokines

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Plant immune responses must be tightly controlled for proper allocation of resources for growth and development. In plants, endogenous signaling peptides regulate developmental and growth-related processes. Recent research indicates that some of these peptides have regulatory functions in the control of plant immune responses. This classifies these peptides as phyto cytokines as they show analogies with metazoan cytokines. However, the mechanistic basis for phyto cytokine-mediated regulation of plant immunity remains largely elusive. We identified GOLVEN2 (GLV2) peptides as phyto cytokines in *Arabidopsis thaliana*. By peptide application, GLV2-precursor overexpression and loss-of-function studies we discovered that GLV2 enhances sensitivity of plants to elicitation with the immunogenic bacterial flagellin epitope flg22. GLV2 is perceived by ROOT MERISTEM GROWTH FACTOR 1 INSENSITIVE (RGI) receptors and RGI3 forms ligand-induced complexes with the flg22 receptor FLAGELLIN SENSITIVE 2 (FLS2), suggesting that RGIs contribute to antibacterial immunity and are part of pattern recognition receptor signaling platforms. GLV2 perception increases posttranscriptional FLS2 abundance and RGIs promote FLS2 protein accumulation. Thus, our data suggest that GLV-RGI signaling controls above ground plant immunity via a previously undescribed mechanism of phyto cytokine activity.

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.

Mono(ADP-ribosyl)ation mediated by a noncanonical ADP-ribosyltransferase counteracts ubiquitination for plant immune regulator homeostasis

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Protein ADP-ribosylation is a covalent and reversible post-translational modification that transfers ADP-ribose from NAD⁺ onto acceptor proteins. Poly(ADP-ribosyl)ation (PARylation), catalyzed by poly(ADP-ribose) polymerases (PARPs) and removed by poly(ADP-ribose) glycohydrolases (PARGs), regulates a wide range of biological and cellular processes. However, the chemistry and physiological functions of mono(ADP-ribosyl)ation (MARYlation) remain unknown. Through a genetic screen in *Arabidopsis*, we reveal that PARylation plays a pivotal role in plant immunity. In this study, we identified two PARG1-interacting zinc fingers (PIZ1/2) proteins, which are key regulators of immune gene expression and positively regulate plant immunity. Immune elicitation promotes PIZ1/2 ADP-ribosylation in a PARG1-dependent, but PARPs-independent manner. PIZ1/2 are MARYlated by plant noncanonical mono-ADP-ribosyltransferase (mART) at multiple glutamate and aspartate residues. Surprisingly, PARG1 has an unconventional activity in hydrolyzing both poly(ADP-ribose) and mono(ADP-ribose) from acceptor proteins. MARYlation of PIZ1/2 antagonizes their polyubiquitination, thereby stabilizing the protein levels to relay immune signaling. Thus, our study uncovers the previously unrecognized enzymes mediating MARYlation of immune regulators, and underpins a unique mechanism of maintaining protein homeostasis by the counter-regulation of ADP-ribosylation and polyubiquitination to ensure proper immune responses.

Activating the plant immune system via detection of soybean cyst nematode proteases

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The soybean cyst nematode (SCN) is the most damaging pathogen of soybean in North America. SCN parasitism is facilitated by effector proteins, which are synthesized in the nematode's esophageal glands and delivered into host cells. Among these effectors are several different proteases. The long-term goal of our project is to create decoy substrates that can be cleaved by these proteases and then activate programmed cell death to inhibit feeding site development. The Innes lab has recently engineered the soybean protein, PBS1-1, to harbor the cleavage site for the NIa protease from the soybean mosaic virus. Transgenic soybean plants that overexpress this decoy protein are completely resistant to the virus. To extend the decoy technology to recognize SCN proteases, we have selected several proteases expressed in SCN glands. Using plant immune suppression assays and host induced gene silencing, we will identify which proteases are indispensable for infection. These proteases will be further characterized by identifying the plant proteins they target using biotin proximity labeling. Cleavage sequences will be identified using an *Escherichia coli*-based genetic screen and inserted into soybean PBS1-1. Functionality of these PBS1-1 decoys will be assessed using

transient assays and in transgenic soybean roots. We expect nematode infection will activate cell death in these roots, which should inhibit the nematode's ability to establish and/or maintain their required feeding site.

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.

TurboID-mediated proximity labeling as novel tool to decipher the molecular mechanisms behind RBP-1 detection and defense activation by the CNL Gpa2

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Plant resistance genes are responsible for specific recognition of pathogens and subsequently activate immune responses. Specific recognition relies on protein-protein interactions for which a variety of techniques has been developed in the past decades, each with its own advantages and limitations. In plants, TurboID-mediated proximity labeling is a novel but powerful approach to detect transient protein-protein interactions in particular, which are harder to detect in conventional approaches such as Co-IP and BiFC1. In *Solanum tuberosum* the single dominant resistance gene *Gpa2* encodes a CC-NB-LRR protein which confers resistance to the potato cyst nematode *Globodera pallida* by recognizing the effector Ran-Binding Protein 1 (RBP1). Despite efforts, a direct protein-protein interaction between these proteins has never been detected suggesting involvement of a third component. Gpa2-mediated cell death depends on the host factor Ran GTPase Activating Protein 2 (RanGAP2), together present in the cell as a preformed complex2. Moreover, RBP1 was recently shown to target the RanGAP2 WPP domain3. A hypothesized model emerges in which these three components form a tripartite complex to facilitate pathogen detection and immune activation that we prompted to test by TurboID-mediated proximity labeling. Here, we present the set-up of the system and its application to resolve the molecular mechanisms underlying RBP1 detection and defense activation by the immune receptor Gpa2.

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.

Structure of the activated ROQ1 resistosome directly recognizing the pathogen effector XopQ

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Plants and animals detect pathogen infection via intracellular nucleotide-binding leucine-rich repeat receptors (NLRs) that directly or indirectly recognize pathogen effectors and activate an immune response. How effector sensing triggers NLR activation remains poorly understood. Structure-function studies of these complexes are hampered by low levels in native tissue, our inability to express them recombinantly, and their instability in solution. We overcame sample limitation problems and solved a 3.8 Å resolution cryo-EM structure of the activated ROQ1, an NLR native to *N. benthamiana* with a Toll-like interleukin-1 receptor (TIR) domain, bound to the *Xanthomonas* effector XopQ. ROQ1 directly binds to both the predicted active site and surface residues of XopQ while forming a tetrameric resistosome that brings together the TIR domains for downstream immune signaling. Our results suggest a mechanism for the direct recognition of effectors by NLRs leading to the oligomerization-dependent activation of a plant resistosome and signaling by the TIR domain.

Glycine-rich RNA-binding protein 7 relies on its intact RNA recognition motif to enhance effector-triggered immunity by Gpa2 and Rx1
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Plant Nucleotide-Binding Leucine-Rich Repeat (NB-LRR) immune receptors often recruit host factors to assist their full functionality, therefore, the identification of NB-LRRs interacting factors is crucial for the further understanding of how NB-LRRs work. In a Co-IP/Mass Spec study, a *N. benthamiana* homologue of the Glycine-Rich RNA-Binding Protein 7 (NbGRP7) is characterized as a novel interactor of potato CC-NB-LRR immune receptor Gpa2, which gives resistance to the potato cyst nematode *Globodera pallida*. GRP7s, which contain an RNA recognition motif at the N terminus, are highly conserved plant proteins involved in RNA processing and have been revealed a potential role in PAMPs-triggered immunity. Interestingly, NbGRP7 also associates with the close homologue Rx1, which encodes a CC-NB-LRR protein but confers resistance to *Potato Virus X* (PVX). Here, we show that NbGRP7 positively regulates effector-triggered cell death by Gpa2 and PVX-triggered extreme resistance by Rx1. Meanwhile, the positive regulation of NbGRP7 in Rx1 function is dependent on its RNA binding capacity, as the mutation on a conserved arginine residue in its RNA recognition motif remains compromises the potentiating effect on Rx1-mediated resistance. Furthermore, we show that NbGRP7 regulates Rx1 transcript levels pre- and post-activation in the cell. Our data demonstrate that NbGRP7 works as a novel pro-immune component in effector-triggered immunity by Gpa2 and Rx1 at the post-transcriptional level.

An integrated approach of molecular dynamics and alanine scanning mutagenesis identifies two aspartic acid residues as responsible for the pH-dependent activity of OG-OXIDASE 1

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After the early-phase of infection, plant cells secrete different OG-oxidases (OGOx) paralogs, defense flavoproteins oxidizing the reducing ends of oligogalacturonides (OGs), a well known class of Damage Associated Molecular Patterns (DAMPs). The oxidized OGs lose their elicitor nature and increased recalcitrance to enzymatic hydrolysis. Among the plant defense responses, the increase of apoplastic pH is hypothesized to be pivotal in reducing the degrading potential of microbial PGs on one side and in boosting the oxidizing activity of OGOx on the other. In accordance with this hypothesis, all OGOx so far characterized show an optimum activity at alkaline pH values. By an integrated approach of molecular dynamics and alanine-scanning mutagenesis, we identified the amino acids D325 and D344 as responsible for the pH-dependent activity of OG-OXIDASE 1 (OGOx1) at basic pH. D325 and D344 act in antagonistic manner in modulating the activity of OGOx1 at pH 8.5 but not at pH 5.0. Compared to the wild-type enzyme, D344A mutation increased OGOx1 activity whereas the D325A mutation reduced it; intriguingly, the activity of OGOx1 variants was altered in opposite manner by a similar extent, suggesting that pH dependent activity of OGOx1 is dependent on the antagonistic action of both amino acids at basic pH.

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.

Evaluation of the efficacy of bio fungicides, plant extracts and chemical fungicides in the management of *Fusarium incarnatum equiseti* species complex infecting bottle gourd

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In the current study, bottle gourd [*Lagenaria siceraria*] plants exhibiting necrotic water soaked lesions on leaves, bark splitting, exudation of gummy ooze, wilting and complete drying of vines were collected and the pathogen was isolated by standard tissue isolation method. The pathogen was morphologically identified as *Fusarium* species by visual observation of macroconidia under microscope. Later, amplification of

ITS (Internal transcribed spacer) region of the conserved rDNA using primers ITS1 and ITS4, sequencing and analysis revealed it as *Fusarium incarnatum equiseti* complex. So far, proper management strategy was not developed for controlling gummy stem blight disease of cucurbits caused by *Fusarium* species. In order to develop integrated management practices, the pathogen was screened against promising biocontrol agents and native bacterial isolates of Tadepalligudem region of West Godavari district of Andhra Pradesh. Strongest inhibition of mycelial growth was shown by *P. fluorescens* (83.83%) followed by native bacterial isolate *P. aeruginosa* (82.47%). In vitro screening with botanical extracts at 2.5%, 5.0% and 10.0% concentrations revealed that maximum inhibition in mycelial growth was exhibited by leaf extract of *Punica granatum* (62.72%). Maximum inhibition of mycelial growth was shown by Carbendazim (100%) followed by Azoxystrobin (81.08%) subsequent to screening of 8 chemical fungicides which were reported to be effective against other *Fusarium* species.

Identification and characterization of protein-protein interactions between RKN effectors and their host targets in the regulation of plant immunity

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Plant-parasitic nematodes produce effectors to overcome plant immunity and fine-tune plant cellular processes. Molecular mechanisms of how effector proteins co-opt plant processes to support nematode survival have been intensively investigated, but they are still poorly understood. Identifying protein-protein interactions is crucial for understanding this cross-kingdom network. Using high throughput screening techniques such as Y2H-seq and TurboID-MS, we have identified tomato proteins involved in various cellular processes interacting with *M. javanica* effectors. Among those, Mj-4D01 was found to interact with heavy metal transport/detoxification superfamily protein and Rieske iron-sulfur protein (ISP), one of the putative subunits of cytochrome b6f complex. We show that Mj-4D01 is involved in suppressing ROS production in plastids. In addition, Mj-4D01 can inhibit INF1-induced cell death. By using the same screening techniques, we have identified the tomato transcriptional factor; TEOSINTE BRANCHED 1, CYCLOIDEA, PCF 15 (Sl-TCP15) as the converging point for several *M. javanica* effectors. Our results show that all the effectors target the Sl-TCP15 DNA binding domain altering its transcriptional profile. This study is an important step closer to understand the dynamic interplay between effector proteins and their plant targets.

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.

How does *Arabidopsis thaliana* fight off its ever-present opportunistic pathogen *Pseudomonas viridiflava*?

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During evolution of host-pathogen interactions, pathogens improve their virulence tools while hosts advance their defenses. *Pseudomonas viridiflava*, an opportunistic pathogen, is the most common *Pseudomonas* clade in European *Arabidopsis thaliana* populations. Belonging to the *P. syringae* complex, it is genetically and phenotypically distinct from well-characterized *P. syringae* sensu stricto. Despite its broad range we lack knowledge of how *A. thaliana* responds to diverse co-occurring *P. viridiflava* strains and how they colonize *A. thaliana*. Here, we characterized the host response in a *A. thaliana*-*P. viridiflava* pathosystem. We measured host and pathogen growth in infections to ascertain gene × gene interactions and used immune mutants and transcriptomics to determine defense pathways influencing infection. We found a large effect of host genotype on infection outcome and evidence of host × pathogen genotype interaction. Immune mutant and transcriptome analyses showed that *A. thaliana* uses jasmonic acid (JA)/ethylene (ET) signaling to defend itself against *P. viridiflava*, more so than salicylic acid. Our results suggest JA/ET is important for suppression of *P. viridiflava*, yet suppression capacity varies between accessions due to still unknown mechanisms. Our results shed light on how *A. thaliana* suppress the ever-present *P. viridiflava*, but further studies are needed to understand how this interaction contributes to persistence of *P. viridiflava* in *A. thaliana* populations.

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.

A phytochemical TIR domain effector cleaves NAD⁺ into a novel cADPR variant to promote virulence

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TIR-NB-LRRs are plant NLRs that cleave NAD⁺ and activate plant immunity. The pathogen *Pseudomonas syringae* secretes a type III effector HopAM1 that that suppresses plant immunity and contains a TIR domain homologous to TIR-NB-LRRs. In vitro and in vivo assays were conducted to determine if HopAM1's TIR domain cleaves NAD⁺ and is essential for HopAM1's suppression of plant immunity and contribution to virulence. HPLC and LC-MS were used to analyze metabolites produced from NAD⁺ by HopAM1 in vitro and in both yeast and plants. *Agrobacterium*-mediated transient expression and in planta inoculation assays were performed to determine HopAM1's enzymatic activity and virulence contribution in plants. HopAM1's TIR domain is catalytically active and cleaves NAD⁺ to produce nicotinamide and a novel cADPR variant we term 'v2-cADPR'. Expression of HopAM1 triggers cell death in yeast and plants in a TIR domain-dependent manner in association with depletion of endogenous NAD⁺. Furthermore, HopAM1's TIR domain is required to suppress both PTI and ETI and promote *P. syringae* virulence. HopAM1 modulates endogenous NAD⁺ to produce v2-cADPR to promote pathogenesis. This work suggests that HopAM1's TIR domain possesses different catalytic specificity than that of TIR domain-containing NLRs and that pathogens exploit this activity to sabotage NAD⁺ metabolism for immune suppression and virulence.

Evidences of *Lilium* defense protein LsGRP1-inducing systemic disease resistance and related long-distance signaling pathways

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Systemic resistance is a critical strategy of plants against pathogen attack. *Lilium* plants emerge systemic resistance to gray mold post *Botrytis elliptica* infection, and *Lilium* defense protein LsGRP1 accumulates in both local, infected and systemic, un-infected leaves, suggesting an involvement of LsGRP1 in systemic resistance induction. In addition, systemic resistance to lily gray mold could be demonstrated by applying LsGRP1 recombinant protein or via agroinfiltration. In *Arabidopsis*, exogenous application of LsGRP1 recombinant protein reduced the population of *Pseudomonas syringae* pv. *tomato* DC3000 in systemic leaves and increased callose deposition post flg22 treatment. Assays of LsGRP1 and its partial deletions in *Arabidopsis* showed that LsGRP1^N and LsGRP1^C mainly involved in the induction of systemic disease resistance. Long-distance signaling pathways, salicylic acid (SA) and pipecolic acid (Pip)-related, for the induction of systemic resistance in *Arabidopsis* were examined upon LsGRP1 treatment and the results showed the reductions of disease symptom and in the systemic leaves of both tested mutants, implicating that SA and Pip long-distance signaling pathways would be related to systemic disease resistance induced by LsGRP1.

Optimizing the PBS1 decoy system to confer resistance to Potyvirus infection in Arabidopsis and soybean

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The Arabidopsis resistance protein RPS5 is activated by cleavage of PBS1 by the *Pseudomonas syringae* effector protease AvrPphB. We have shown replacing seven amino acids at the cleavage site of PBS1 with the cleavage motif of the turnip mosaic virus (TuMV) NIa protease enables RPS5 activation upon TuMV infection. However, this confers a trailing necrosis indicative of a cell-death response too slow to contain the virus. We theorized this could be due to a positional mismatch within the cell between PBS1TuMV, RPS5, and the NIa protease. To test this, we relocated PBS1TuMV and RPS5 to cellular sites of NIa accumulation, revealing relocation of RPS5 compromised RPS5-dependent cell death in *Nicotiana benthamiana*, even though PBS1 was efficiently cleaved. Alternatively, we tested if overexpression of PM-localized PBS1TuMV could enhance immunity to TuMV. Significantly, this conferred complete resistance to TuMV delivered by agrobacterium or via aphid transmission, showing RPS5-mediated defense responses are effective against bacterial and viral pathogens. The PBS1 decoy approach was also extended to soybean by modifying a GmPBS1 ortholog to be cleaved by the soybean mosaic virus (SMV) NIa protease. Transgenic overexpression of the native PBS1 decoy conferred immunity to SMV, demonstrating that we can use endogenous PBS1 proteins in crops to engineer economically relevant disease resistant traits.

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.

A cell wall degrading enzyme and its putative receptor/co-receptor activate rice and Arabidopsis immune responses via different signaling pathways

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Plant pathogens secrete various cell wall degrading enzymes (CWDE) to degrade different components of the plant cell wall. Plants can perceive damage caused by these CWDE and mount immune responses (IRs). But how do plants perceive it and mount immunity is not well explored. Lipase/esterase (LipA) is a CWDE that is secreted by *Xanthomonas oryzae* pv. *oryzae* (Xoo), the bacterial blight pathogen of rice. Treatment of rice or Arabidopsis tissue with LipA or LipA treated cell wall extract induces IRs. Using transcriptome analysis, we identified a rice receptor kinase *OsWAKL21* which is upregulated following treatment of rice leaves with LipA or Xoo but not after treatment of LipA mutant of Xoo. Downregulation of *OsWAKL21* in rice attenuated LipA induced IRs. Overexpression of *OsWAKL21* in rice mimics LipA treatment in terms of IRs. Ectopic expression of *OsWAKL21* in Arabidopsis also induces similar IRs. *OsWAKL21* is a moonlighting kinase that has kinase and guanylate cyclase (GC) activities. Interestingly, kinase activity of *OsWAKL21* is required for induction of rice IRs while in Arabidopsis it needs its GC activity. The expression profile of defense genes and experiments with mutant Arabidopsis lines indicate LipA treatment or overexpression of *OsWAKL21* induces JA pathway in rice while it acts via SA pathway in Arabidopsis. Thus, our results indicate that LipA and its putative receptor/co-receptor *OsWAKL21* activate rice and Arabidopsis immunity via two different signaling pathways.

Identification of novel immune-suppressing effectors from the wheat pathogen *Zymoseptoria tritici*

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Zymoseptoria tritici is the major fungal pathogen of wheat in Europe. Despite its significance, very little is known about how *Z. tritici* is able to induce disease and how it evades host defenses during its extended latent/biotrophic growth phase inside the wheat leaf. Aside from well-characterized LysM domain effectors that mask the fungus' chitin from the host, *Z. tritici*'s mechanisms of host immune suppression are poorly understood. We have performed different screens of *Z. tritici* effectors in both *Nicotiana benthamiana* and wheat, and have identified effector candidates as immune-suppressors. To date, we have primarily focused on effectors able to suppress BAK1-triggered immunity. *Z. tritici* has two homologues of the BAK1-interacting effector NIS1. One of these homologues is highly expressed during the biotrophic growth phase, and so we have used it as a positive control for immune suppression. At this stage, we have identified an additional four putative BAK1-suppressing effectors among the first 12 effectors screened. Each of these effectors are capable of suppressing *Pseudomonas syringae*-induced cell-death in *N. benthamiana*. In summary, our studies have identified novel effectors used by *Z. tritici* to suppress host immune responses. This is part of an ongoing project to dissect the wheat-*Z. tritici* molecular interactions, with a long-term goal of identifying mechanisms of host-specificity.

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.

Functional characterization of SIPBS1 to broaden the recognition specificity of an NLR protein in tomato

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In *Arabidopsis*, resistance protein RPS5 is specifically activated by sensing the status of a decoy protein, PBS1, which is cleaved by AvrPphB from *P. syringae*. The recognition specificity of RPS5 was successfully expanded by substituting AvrPphB cleavage sequence within PBS1 to other new proteases from different pathogens in *Arabidopsis*. Here, we translate our knowledge of PBS1 decoy engineering to tomato. Remarkably, delivery AvrPphB into tomato (*Solanum lycopersicum*) induces a strong defense response suggesting that tomato may possess an *Arabidopsis* RPS5 ortholog to recognize AvrPphB. Moreover, an Arabidopsis PBS1 ortholog in tomato, SIPBS1, can be cleaved by AvrPphB. Significantly, the engineered SIPBS1 harboring TEV or PVY NIa protease cleavage sequence can also be cleaved by the corresponding proteases. This observation suggests a possible application of the novel 'decoy' system into tomato to engineer disease resistance against economically important pathogens with cysteine protease activity. In addition, we are generating *slpbs1* knock-out mutant plants with CRISPR/Cas system to characterize the *SIPBS1* function. Our approach contributes a significant impact on current strategies for developing disease-resistant crops.

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

The role of auxin in the virulence of the cacao pathogen *Moniliophthora perniciosa*

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The cacao tree (*Theobroma cacao*) is a culture of great socioeconomic importance in many tropical countries. A major limiting factor for cocoa production in Latin America is witches' broom disease (WBD), caused by the basidiomycete *Moniliophthora perniciosa*. Plants affected by WBD show intense morphological changes and develop abnormal shoots, which indicates the occurrence of hormonal imbalances during the infection. Yet, the contribution of specific plant hormones to such changes remains poorly explored. Using dual-RNA-seq, we identified a remarkable up-regulation of auxin-responsive genes in infected plants. However, cacao genes involved in auxin production were not activated, suggesting that the auxin response observed in infected plants is triggered by the pathogen. Investigation of the *M. perniciosa* genome revealed putative pathways for indole-3-acetic acid (IAA) production. Indeed, supplementation of *M. perniciosa* cultures with the IAA precursor tryptophan induced the accumulation of indole compounds. We are now validating the predicted IAA biosynthetic pathways in this pathogen. Using CRISPR/Cas9, we will investigate the role of IAA in fungal virulence and in WBD symptom development. We will specifically evaluate if IAA interferes with the host immune capacity. In addition to exploring how *M. perniciosa* induces morphological changes in cacao, this work may also expand our understanding of how phytohormone production by fungal pathogens promote plant susceptibility.

The flax-rust AvrP effector protein appears to be a bifunctional effector targeting both glucose and RNA metabolism in flax

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Rust diseases are a serious threat to cereals and other crops worldwide. The interaction between flax and the flax rust fungus, *Melampsora lini*, is a model pathosystem helping us to understand the molecular basis of rust fungal pathogenicity in plants. The AvrP effector protein is translocated into the plant cell by the pathogen to facilitate infection. To investigate the role of AvrP, plant interactors were identified via a yeast two hybrid (Y2H) screen and confirmed by in planta assays. AvrP was found to interact with flax phosphoglucosyltransferase and DEAD Box RNA Helicase 56 (DRH56). Further Y2H analysis showed that AvrP targets the phosphate binding domain of phosphoglucosyltransferase. In an *in vitro* enzymatic assay, purified AvrP protein was able to modulate phosphoglucosyltransferase activity for increased glucose 6-phosphate production. Bimolecular fluorescence complementation analysis showed that AvrP and DRH56 interact inside plant nuclei and co-expression of AvrP and DRH56 in *Nicotiana benthamiana* leaves resulted in cell death. Mutational disruption of DRH56 ATPase activity did not abolish its interaction with AvrP nor their ability to induce cell death in *N. benthamiana*. Further Y2H analysis showed that AvrP interacts with conserved motifs involved in RNA binding and helicase activity. Overall, these results suggest that AvrP is a bifunctional effector able to affect both glucose and RNA metabolism by manipulation of flax phosphoglucosyltransferase and DRH56, respectively.

Homologous and functionally distinct effectors differentially target NLR immune receptor RB

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Significant progress has been made in the molecular basis of plant nucleotide-binding leucine-rich repeat (NLR) immune receptor-mediated recognition of their corresponding effectors and activation of immune responses, but how these processes are overcome by pathogens remains to be elucidated. The nucleotide-binding leucine-rich repeat (NLR) immune receptor RB confers broad-spectrum resistance against *Phytophthora infestans* by recognizing the prevalent IPI-O family effectors (e.g., IPI-O1). The processes can be suppressed by an infrequently present homologous effector IPI-O4. Here, we report that, despite high homology, the avirulence effector IPI-O1 and virulence effector IPI-O4 differ strikingly in subcellular localization, the status of self-association, and impacts on the self-association of the RB CC domain. Furthermore, IPI-O4 disrupts IPI-O1 self-association but enhances the interaction between RB CC and IPI-O1. Our results point to a previously unrecognized mechanism in which the virulence effector IPI-O4 suppresses NLR RB-mediated recognition of its cognate effector IPI-O1 via disrupting the self-association of both RB CC and IPI-O1.

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.

The TGA transcription factors negatively regulate the accumulation of the defense hormone salicylic acid

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Salicylic acid (SA) is a hormone that modulates changes in gene expression, inducing the defense response in plants. The mechanisms that control the accumulation of SA are essential to understand the defensive process. The TGA class II transcription factors (TF) that include the proteins TGA2, 5, and 6 are crucial mediators for the transcription of SA-controlled genes. We found that plants lacking these TFs (*tga256* plants) showed higher expression of genes involved in SA accumulation than WT plants. To corroborate the participation of TGA class II on the SA-biosynthetic pathway, we induced the SA accumulation through the treatment with UV-C radiation and inoculation with the pathogen *Pseudomonas syringae* AvrRPM1. After stress, the *tga256* plants showed an increased expression of genes involved in SA biosynthesis and an increase in the SA content as well. The expression of TGA2 in the *tga256* genetic background rescued the phenotype to a WT state. We also found that the SA over-accumulation on *tga256* plants depends on the Isochorismate pathway. Together, these results suggest that TGA class II TFs negatively control SA production under stress conditions. In spite of this group of TF has been described as players on the defense response, they have never been associated with the SA-biosynthetic pathway. Even when these proteins are *old dogs* in the defense response context, we still discovering new tricks. Financed by FONDECYT iniciación 11200944, Regular 1210320 and ICM iBio.

Leveraging the natural diversity of the Arabidopsis TIRome for structure-function insights into TIR-mediated resistance

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Toll/Interleukin-1 Receptor (TIR) domain proteins are encoded by species within all domains of life, and typically play roles in cell fate decisions and immunity. In dicot plants, TIR-domains fused to a modular NBS-LRR (nucleotide-binding site-leucine-rich repeat) chassis form a major class of disease resistance (*R*) proteins (TNLs). While most TIR-domains of animals are reported to form signaling scaffolds, recent findings indicate that the exceptional animal TIR protein SARM1, and multiple prokaryotic and plant TIR-domains possess NAD⁺-hydrolase activities, which are essential for signaling. Importantly, how diverse plant TIR NADases activate different immune outputs and signal to downstream mediators is unclear. Although numerous TNLs from Arabidopsis have been investigated, many TNLs—as well as many TIR-domain containing proteins of atypical architectures—remain phenotypically uncharacterized; and considerable sequence diversity exists among these TIRs. Using transient assays in *Nicotiana* spp., we are phenotyping all predicted TIR-domains of Col-0 for their ability to trigger auto-active cell death. Certain features of plant TIRs, such as TIR-TIR association interfaces and a putative catalytic glutamate, are critical for NADase functions and immune signaling. Through screening the large diversity of Col-0 encoded TIRs, we anticipate identifying additional features of TIR-domains which also contribute to immune signaling.

***Xanthomonas euvesicatoria* gene Xc4429 encodes a protein functions as an antitoxin, a transcription repressor, and an enhancer of XcAvrRxo1 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 *Xc4428* encodes a type III effector (T3E) that shares 89.67% amino acid identity with *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) T3E *AvrRxo1*. Deletion of *Xc4428* in the genome of *X. euvesicatoria* (strain Xcv85-10) compromised its virulence to infect pepper and *Nicotiana benthamiana* plants. Transient co-expression of *Xc4428* and *Rxo1* on pepper and *N. benthamiana* plant leaves results in a strong hypersensitive reaction. Thus, *Xc4428*, renamed as *XcAvrRxo1*, is a bona fide orthologue of *XocAvrRxo1* that possesses both virulence and avirulence functions. Expression of *XcAvrRxo1* is toxic in *E. coli* and *X. euvesicatoria* cells. Another *X. euvesicatoria* gene *Xc4429*, encodes a putative chaperone of *XcAvrRxo1*, which can interact with *XcAvrRxo1* to suppress its toxicity in *X. euvesicatoria* and *E. coli* bacterial cells. *Xc4429* also binds to the promoter region of *XcAvrRxo1* and represses its transcription in *X. euvesicatoria* bacterial cells. In addition, expression of *Xc4429* can enhance the secretion and translocation of *XcAvrRxo1* into plant cells. Therefore, *Xc4429* functions as an antitoxin, a transcription repressor, and a type III chaperone that is capable of enhancing the secretion and translocation of *XcAvrRxo1* during pathogenesis.

Identification of a gene cluster encoding at least two effector proteins involved in host-specificity of *Sporisorium reilianum*

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Sporisorium reilianum is a biotrophic fungus and the causal agent of head smut in sorghum and maize. Two different formae speciales, *S. reilianum* f. sp. *reilianum* (SRS) and *S. reilianum* f. sp. *zeae* (SRZ), can specifically infect sorghum or maize, respectively. Both formae speciales were shown to be closely related on the genomic level, however, the characteristic features responsible for their host-adaption remain unknown. In a classical genetics approach, we identified a gene cluster consisting of nine genes, predicted to encode secreted effector proteins, whose presence highly correlated with virulence on sorghum plants. This cluster was previously annotated as divergence cluster 7_11 between *S. reilianum* and *Ustilago maydis*. The deletion of cluster 7_11 in SRS resulted in reduced spore formation in the inflorescences of sorghum plants. In addition, plants infected with SRS cluster 7_11 deletion strains showed phytoalexin accumulation in leaves, a characteristic sorghum defence response against SRZ infection. We identified two specific cluster genes that are involved in the reduction of virulence and phytoalexin induction, respectively. Therefore, cluster 7_11 contains at least two genes important for host-specificity of *S. reilianum*.

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 cassiicola* 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 induce 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.

Identification of a putative DNA-binding protein in Arabidopsis that acts as a susceptibility hub and interacts with multiple *Pseudomonas syringae* effectors

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Phytopathogens suppress host immunity and promote pathogen virulence using secreted effector proteins. There is increasing evidence that the host-pathogen interactome comprises a complex network. Previous large-scale interaction screens did not include *Pseudomonas syringae* effector HopZ1a, a member of the YopJ superfamily of effector proteins found in animal and plant-infecting bacteria. To identify novel interactors of HopZ1a, we performed a yeast two-hybrid screen that identified a previously uncharacterized Arabidopsis protein that we call HopZ1a Interactor 1 (ZIN1). Additional analyses in yeast and *in planta* showed that ZIN1 also interacts with several other *P. syringae* effectors. We demonstrate that a loss-of-function *zin1* mutant is less susceptible to infection by certain strains of *P. syringae*, while overexpression of *ZIN1* results in enhanced susceptibility. Functionally, ZIN1 exhibits topoisomerase-like activity *in vitro*. We carried out transcriptional profiling of wild-type and *zin1* Arabidopsis plants inoculated with *P. syringae*. While ZIN1 regulates a wide range of pathogen-responsive biological processes, the list of genes more highly expressed in *zin1* versus wild-type plants is particularly enriched for ribosomal protein genes. Taken together, these data reveal ZIN1 as a potential susceptibility hub that interacts with multiple effectors to influence plant-microbe interactions.

The C-terminal domain of the *Ralstonia solanacearum* type III effector RipY is required for recognition in *Nicotiana benthamiana*

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Ralstonia solanacearum (Rso) is a causal agent of the devastating bacterial wilt disease in solanaceous crops. This pathogen injects around 70 effectors into host cells to promote infection. In turn, effectors, or their activities are monitored by plant nucleotide-binding leucine-rich repeat receptors (NLRs), a major class of resistance proteins. NLR activation leads to restriction of bacterial multiplication, often associated with a hypersensitive response (HR) and confers disease resistance. Here, we found that expression of different alleles of the Rso effector RipY induced rapid cell death in *Nicotiana benthamiana* (Nb). This cell death was dependent on the NLR-chaperone SGT1, indicating that RipY could be recognized by an NLR in Nb. Moreover, delivery of RipY by the virulent *Pseudomonas syringae* pv. *tomato* DC3000 *hopQ1-1* mutant restricted the bacterial growth, suggesting that RipY recognition might confer efficient pathogen resistance. RipY is predicted to harbor a putative type III secretion signal, an α -helix domain, repetitive ankyrin motifs, and an alanine (A)-glutamic acid (E) rich region. RipY truncations lacking each putative domain/motif were constructed to determine the region required for the effector recognition. We found that the expression of several C-terminal truncations could not trigger HR in Nb. Overall, our data indicated that the C-terminal region of RipY is required for the activation of a matching NLR that confers resistance responses in Nb.

The NLR-ID TaAdnr1 functions in the recognition of aphid infestation

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Plants respond in a similar manner to pest infestation as to pathogen ingress. An economically important aphid is *Diuraphis noxia* that is a highly specialized aphid pest on a limited number of cereals and grasses. Wheat cultivars, carrying the *Dn*-resistance genes that imparts resistance

against this pest, has driven the development of several resistance breaking biotypes with five occurring in South Africa. When these aphid biotypes feed on wheat, they induce similar phenotypic responses to those observed during pathogen infection. To date none of the underlying wheat resistance genes have been identified, but a WRKY integrated domain nucleotide-binding leucine-rich repeat (NLR-ID) protein (TaADNR1) plays a significant role in detection *D. noxia* infestation. Knockdown studies using VIGS in a *Dn1* (resistant to the RWASA1 biotype) and *Dn7* (resistant to all five RWASA biotypes) carrying wheat cultivar, showed that the loss of this protein result in increased aphid fitness and a highly susceptible wheat phenotype. This observation for four different biotypes of *D. noxia* indicated that biotypification is not based on the loss of recognition by TaADNR1. The integration of the WRKY domain onto the NLR core protein is a very recent *Triticum* specific event as this WRKY domain only occurs in *T. aestivum* and *T. urartu*, where it functions in rust resistance recognition. This hints at a central role of these NLR-IDs in wheat resistance responses.

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.

Understanding mechanisms of nonhost resistance through a genetically tailored Arabidopsis-Powdery mildew pathosystem

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Nonhost resistance (NHR) is effective immunity of a plant species against any non-adapted but potentially pathogenic microbes. However, the molecular basis of NHR remains poorly characterized. Here, we report the construction of a tailored pathosystem that has enabled facile and saturated genetic screens for novel genes contributing to NHR. We first constructed an Arabidopsis *eds1pad4sid2* (*eps*) mutant that is defective in post-penetration resistance. While *eps* plants are fully susceptible to sow thistle PM which is arrested after penetration in all Arabidopsis accessions tested, they are still immune to strawberry PM, suggesting that *eps* still possesses effective penetration resistance against this non-adapted PM. Indeed, knocking out *PEN2* involved in penetration resistance in *eps* rendered the *eps-pen2* mutant susceptible to strawberry PM. We thus conducted saturated genetic screens using EMS-mutagenized *eps* plants challenged with strawberry PM for “*snap*” mutants that are “susceptible to non-adapted PM”. We obtained 36 *snap* mutants and identified 12 independent causal mutations. Among them, 7 are novel mutations in *PEN2*, *PEN3*, or *PEN4*, thus validating the extremely high efficiency of this tailored pathosystem. Importantly, we have identified five novel *SNAP* genes involving in penetration resistance. *SNAP1* encodes a chromatin remodeler, hence, indicating an essential role of epigenetic regulation of genes involved in penetration resistance. More detail will be discussed.

A new *Solanum* LRR-RLK recognizes Pep-13/25 of *Phytophthora*

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The late blight disease caused by the oomycete *Phytophthora infestans* is the major threat for potato production. An important source of immunity relies on pattern recognition receptors (PRR) that are located on the cell surface and recognize microbe-associated molecular patterns (MAMPs). Pep-13/25 is a well-characterized oomycete MAMP, derived from a transglutaminase that is present in all *Phytophthora* species, including *P. infestans*. Infiltration of Pep13/25 peptides leads to a cell death response in various *Solanum* species. To identify the PRR of Pep13/25, a genetic mapping approach was conducted and combined with BSR-Seq analyses. Further fine-mapping resulted in a 552 kb region containing 3 candidate genes that resemble typical extracellular receptors. One of the candidate genes led to a Pep-13/25 response in transient expression assays, and stable transgenic plants confirmed these results. This gene encodes a protein of 1,125 amino acids that contains a signal peptide, 18 extracellular LRR domains, a transmembrane domain and a cytoplasmic kinase domain. Exploiting the PRR and stacking them with other PRR or *R* genes can provide a more profound strategy to achieve a more durable resistance to late blight in potato.

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.

The role of purine metabolism and extracellular DNA in plant endophyte interaction

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Purine nucleotides and deoxynucleotides are important metabolites in many organisms. They provide cofactors for the central metabolism, function as source of energy and building blocks of nucleic acids. In plants, purine metabolism was only scarcely investigated with focus on intracellular metabolism. Recently, it was found that the mutualistic root endophyte *Serendipita indica* interferes with the apoplastic purine metabolism of its host plants upon colonization. Some purine nucleotides such as ATP are well-characterized microbe associated molecular patterns (MAMPs) and known to induce plant immunity. Studies suggest that also extracellular DNA plays an important role in plant microbe interactions. On the one hand, it seems to play a role in root cap protection by active secretion of DNA and other components to entrap and kill pathogens, a similar mechanism is known from mammalian immune cells. On the other hand, self-DNA might be sensed as a DAMP and trigger immune responses such as accumulation of reactive oxygen species (ROS) and growth inhibition. Here, I investigate the role of extracellular DNA and purine nucleotides in plant-microbe interactions using the model plant *Arabidopsis thaliana* and the crop *Hordeum vulgare* in light-emission based assays to monitor calcium influx and ROS accumulation to examine whether DNA and purines can be sensed as DAMPs and MAMPs.

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.

MORC1 interacts with a bZIP transcription factor and positively regulates plant immunity against *Phytophthora infestans* in Solanaceae

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Phytophthora infestans is a destructive oomycete pathogen that was responsible for the Irish potato famine and remains a global threat for food security. Previous studies have demonstrated that members of the GHKL ATPase superfamily called microorchidia (MORC) act as key players during plant immunity against different kind of pathogens, including *P. infestans*. This study aims to identify MORC1-interactors (MIPs) in Solanaceous crops using biochemical, genomics, and proteomics approaches to determine their role during plant immunity. Through a yeast two-hybrid screening, a basic leucine zipper (bZIP) transcription factor (MIP10) was found to physically interact with MORC1. Interestingly, this interaction was driven by their C-terminal regions. Similar to MORC1, MIP10 lacks a nuclear localization signal, and yet its abundance in the nucleus is enhanced upon flagellin 22 treatment. Silencing MIP10 in *Nicotiana benthamiana* enhanced pathogen susceptibility and compromised cell death induced by pathogen-associated molecular pattern (PAMP)-triggered immunity (PTI) and effector-triggered immunity (ETI). Transient overexpression of tomato *MIP10*(*SIMIP10*) in *N. benthamiana* enhanced pathogen resistance. Finally, silencing *SIMIP10* in tomato compromised ETI induced by *Pto-AvrPto* recognition. Together, these results argue that this bZIP transcription factor is a positive regulator of plant immunity and can be used to engineer *P. infestans* resistant crops.

A self-compartmentalized enzyme complex controls NLR activation in plants

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The multigene locus *DM2* in *Arabidopsis thaliana* encodes TIR-NLRs with high sequence diversity. Numerous *DM2* genes trigger autoimmunity in different genetic backgrounds; in particular *DM2h* from the Bla-1 accession results in autoimmunity in the presence of *DM3* from Hh-0. *DM3* encodes an uncharacterized protein with the alpha/beta hydrolase fold and discernible catalytic triad, which are the features found in serine proteases or O-acetyltransferases. To understand molecular mechanisms underlying *DM2h* NLR activation in the presence of the particular *DM3* variant, we determined the cryo-EM structure of *DM3* from Col-0 at 3.76Å resolution. This high-resolution cryo-EM structure revealed that the six *DM3*s form a stable self-compartmentalized chamber with the small openings at the top and the bottom, which is reminiscent of proteasome. In this structure, the catalytic sites are located inside of the chamber and the access to the sites are limited from outside. Interestingly, two residues specific to *DM3^{Hh-0}* are located at the interface of *DM3* oligomerization. Our thermal shift assay shows that the stability of the self-compartmentalized structure of *DM3^{Hh-0}* is significantly compromised. Structure guided mutations to disrupt the oligomeric structure and thermal shift assay demonstrated that the stability of the complex is directly associated with the *DM2h* NLR activation in plants. Ongoing works on assessing the role of *DM3* in plant immunity will be further discussed.

Coordinated regulation of plant immunity by poly(ADP-ribosylation) and K63-linked ubiquitination

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Poly(ADP-ribosylation) is a post-translational modification catalyzed by poly(ADP-ribose) polymerase (PARP) and plays a key role in multiple cellular processes, but its function in plant immunity is largely elusive. A yeast two-hybrid screen using *Arabidopsis* PARG2 as bait identified UBC13B, an ubiquitin E2 conjugating enzyme, which is a major driver of K63 polyubiquitin chain formation. Co-immunoprecipitation assays revealed that both UBC13A and UBC13B interact with PARP1/2 and PARG1/2 in vivo. Flg22, which acts as a pathogen-associated molecular pattern (PAMP), promotes these interactions and enhances poly(ADP-ribosylation) of UBC13B. Bacterial growth assays showed that the *parp1/parp2* and *ubc13a/ubc13b* mutants are more susceptible to the bacterial pathogen *Pseudomonas syringae* compared to WT plants. Intriguingly, the accumulation of total pathogenesis-related (PR) proteins increased in the *parp1/parp2* and *ubc13a/ubc13b* mutants but the levels of secreted PR proteins decreased in these mutants compared to WT. Furthermore, a number of proteins involved in secretory pathway, such as protein disulfide-isomerases (PDIs), complex with PARP1/2 and PARG1/2 and are poly(ADP-ribosylated) upon PAMP treatment. Poly(ADP-ribosylation) of UBC13 and PDIs facilitates K63-linked ubiquitination of PDIs and promotes their disulfide isomerase activities. Our results suggest that plant immunity is coordinately regulated by poly(ADP-ribosylation) and K63-linked ubiquitination.

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.

Tiny things considered: Bacterial EVs and plant infection

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Vesiculation is a process employed by Gram-negative bacteria to release extracellular vesicles (EVs) for quickly adapting to changing environments. Being underexplored, we focus on the role of EVs from bacterial pathogens in shaping the interactions with plants. Here, we describe EV production in plant-pathogenic *Pseudomonas syringae* pv. *tomato* DC3000 (*Pto* DC3000), the causal agent of bacterial speck disease. *Pto* DC3000 produced EVs in culture, observed as outer membrane vesicles (OMVs), and in planta, which increased over the time of infection. The potential contribution of EVs to *Pto* DC3000 plant infection was assessed using plant treatments and proteomic analysis of EVs. While these results identify immunogenic activities of the EVs, inducing prototypic immune responses, they did not promote antibacterial immunity. Instead, analysis of the EV-enriched proteins point at roles for vesiculation in bacterial defenses and nutrient acquisition. Since the EVs were enriched proteins functioning in transport i.e. siderophore transport, we explore the EV ionome. These findings expand our understanding how bacterial pathogens achieve plant infection.

Physiological relevance of Fibronectin type 3 domain association with CellobiosidaseA, a virulence factor of *Xanthomonas oryzae* pv. *oryzae*
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Xanthomonas oryzae pv. *oryzae* (*Xoo*) causes bacterial blight disease of rice. *Xoo* uses several cell wall degrading enzymes as virulence factors. CellobiosidaseA (CbsA) is an important enzyme, which is required for the complete virulence of *Xoo*. CbsA is shown to be the factor, which is required for xylem pathogenesis. It has an N-terminal catalytic domain (exoglucanase), which belongs to the GH6 family, and a C-terminal Fibronectin type 3 (FN3) domain. The secreted CbsA lacks FN3 domain and this truncated form is biochemically active and can induce immune response on treatment of rice tissue with purified protein. Interestingly, a deletion mutant of FN3 domain is virulence deficient and shows almost 95% reduction in secreted CbsA level. Further, we have shown that this FN3 domain is required for interaction with cytoplasmic chaperon SecB. This interaction is critical for stability and efficient transport of the protein across the cytoplasmic membrane. We have shown that presence of FN3 domain inhibits the activity of protein on polymeric substrate carboxymethylcellulose, which could be the reason why FN3 domain is absent in the secreted CbsA.

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 5,681 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 Transitoria_FCT-DL57/2016/CP1361/project CT0011 (www.sciencedirect.com/science/article/pii/S0300908420300365?via%3Dihub#gs15).

The secreted peptide IRP functions as a DAMP in rice immunity

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Small signaling peptides play important roles in various plant processes, but information regarding their involvement in plant immunity is limited. We previously identified a novel small secreted protein in rice, named immune response peptide (IRP), whose overexpression in rice suspension cells induced expression of the defense gene *PAL1* and MAPKs activation. IRP was secreted into the apoplast upon treatment with the fungal cell wall component chitin. Here, we studied IRP functions in rice immunity. Rice plants overexpressing *IRP* displayed increased resistance to the blast fungus. Application of the IRP peptide to rice suspension cells triggered the expression of *IRP* itself and *PAL1*. Eighty-four percent of genes upregulated by IRP peptide were also induced by chitin, including 13 *OsWRKY* transcription factors, indicating that IRP and chitin share a similar signaling pathway. Co-treatment with chitin and IRP peptide elevated the expression level of *PAL1* and *OsWRKYs* in an additive manner. When results of *IRP* and *PAL1* expression and MAPK activation by IRP were compared with those by chitin, IRP had a stronger effect on MAPK activation rather than *IRP* and *PAL1* expressions. Collectively, our findings indicate that IRP functions as a damage-associated molecular pattern (DAMP) in rice immunity, regulating MAPKs and *OsWRKYs* to amplify chitin signaling, and provide new insights into how PAMPs and DAMPs cooperatively regulate rice immunity.

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.

Development of jasmonic acid signaling pathway biosensor for following plant immune response in potato (*Solanum tuberosum* L.)

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The spatiotemporal dimensions of plant's response to pathogen invasion determine resistant, tolerant or susceptible phenotype. The main three hormones that are involved in the spread of the immune response are salicylic acid, ethylene and jasmonic acid (JA). We could follow them with

biosensors such as transcriptional reporters, which are based on a promoter sequence that contains analyte-responding *cis*-elements and drives transcription of the reporter gene. However, due to the intertwined pathways of these three hormones, the development of specific sensors is demanding. We are developing a transcriptional reporter of JA signaling pathway in potato. Transcriptomic data led to the selection of two families of JA-responsive genes. Their promoter regions were amplified from genomic DNA of potato genotypes Rywal, Désirée and PW363 according to homologue gene promoters in the genome of Group Phureja clone. We compared the frequencies of the known *cis*-elements involved in biotic stress response and JA signaling pathway among all known *cis*-elements in the identified sequences, and thus selected the most promising candidates for the biosensor. The promoter candidates were fused with firefly luciferase or fluorescent protein coding sequence and transiently expressed in *Nicotiana benthamiana* to select one promoter candidate per gene for the production of biosensoric stable transformants of potato. The work now continues with testing specificity of the promoter to JA-triggered response.

Impaired cuticle functionality and robust resistance to *Botrytis cinerea* in *Arabidopsis thaliana* plants with altered pectin integrity

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The plant cell wall (CW) is a complex structure that acts as a mechanical barrier, restricting the access to most microbes. Altered pectin composition in *Arabidopsis* plants expressing a fungal polygalacturonase (PG plants) that degrades homogalacturonan (HG), as well as mutants for *QUASIMODO2* (*QUA2*), encoding a putative pectin methyltransferase important for HG biosynthesis, leads to accumulation of reactive oxygen species (ROS) and almost complete resistance to the fungal pathogen *Botrytis cinerea*. The exact mechanism linking loss of pectin integrity and fungal resistance is not clear. We found that plants with altered HG have greatly increased cuticle permeability, and both permeability and resistance to *B. cinerea* are suppressed by abscisic acid. Increased cuticle permeability, accumulation of ROS and resistance to *B. cinerea* are also observed in mutants lacking a functional FERONIA, a receptor-like kinase thought to monitor pectin integrity. In contrast, mutants defective in other primary CW components do not show defective cuticle and are normally susceptible to *B. cinerea*. Notably, increased cuticle permeability and fungal resistance in *qua2* are partially dependent on *AtPRX71*, encoding a class III peroxidase that also contributes to ROS accumulation in the mutant. Our results suggest that peroxidase-mediated defects in cuticle integrity play a major role in the robust resistance to *B. cinerea* of plants with altered HG integrity.

Differential immune regulatory functions of BSKs in *Arabidopsis thaliana*

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Accumulating evidence point to a nexus between brassinosteroid signaling kinases (BSK) and plant immunity. The *Arabidopsis* ETI receptor RPS2 forms a protein complex with BSK1, BSK8 and the PTI receptor FLS2. BSK1 was also reported to play a role in PTI. Our phylogenetic analysis of 1,079 BSK sequences from 249 land plants revealed that BSK1 and BSK8 form separate clades that diversified around the time of fern divergence, suggesting potentially distinct functions for BSK1 and BSK8 and its close homolog BSK7. After confirming that both BSK1 and BSK8 physically interact with RPS2 and FLS2 by BiFC and coIP, we analyzed the roles of BSK1, BSK7, and BSK8 in immunity using their combinatorial mutant genotypes. BSK1 had a major positive effect on FLS2-mediated PTI measured by the *Pto* growth inhibition after flg22 treatment. A 3-way positive interaction between BSK1, BSK7 and BSK8 was also observed in FLS2-mediated PTI. BSK1 was also important for FLS2-mediated ROS production while BSK7 and BSK8 were not. In RPS2-mediated ETI, BSK7 and BSK8 together had a significant positive effect on ETI while BSK1 did not. Nonetheless, in RPS2-mediated ETI-specific cell death, functional redundancies were evident not only between BSK7 and BSK8 but also between BSK1 and BSK8. In conclusion, while the physical interaction patterns of BSK1 and BSK8 with RPS2 and FLS2 are alike, the effects of BSK1 and BSK7/8 on PTI and ETI are distinct, highlighting their differential immune regulatory functions.

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.

The tomato receptor-like cytoplasmic kinase BSK830 plays a role in PTI signaling

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Receptor-like cytoplasmic kinases (RLCKs) participate in signaling of plant immunity by transducing signals perceived by pattern-recognition receptors (PRRs). To assess the involvement in immunity of tomato brassinosteroid signaling kinases (BSKs), which are members of the RLCK subfamily XII, we analyzed their physical association with PRRs. BSK830 was found to interact with the tomato FLS2, FLS3, and Bti9 PRRs, and its role in immunity was further investigated. Tomato *bsk830* mutant and overexpression plants displayed enhanced susceptibility to *Pseudomonas syringae* (*Pst*) bacteria and to the fungus *Botrytis cinerea* (*Bc*). In plants with altered BSK830 expression, pattern-triggered immunity (PTI) responses, including ROS accumulation and increase in cytosolic Ca^{2+} , were partially compromised and pretreatment with non-pathogenic bacteria failed to protect from *Pst* and *Bc* infection. Analysis of BSK830 subcellular localization revealed that at the resting state BSK830 evenly distributes at the plasma membrane, while upon flg22 and flgII-28 treatment it is redistributed into puncta and internalized. Together, our findings demonstrate that BSK830 regulates a subset of PTI responses, and its localization is modulated by flagellin sensing. Ongoing investigation will determine the route and functional consequences of BSK830 endomembrane trafficking.

Identification and characterization of RCR1, a gene for resistance to clubroot disease in Arabidopsis

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Clubroot, caused by *Plasmodiophora brassicae*, is one of the most serious diseases of *Brassica* species and causes significant yield losses worldwide. Although two clubroot resistance genes have been cloned and several additional resistance loci have been identified, molecular mechanisms of defense responses against *P. brassicae* remain poorly understood. Here, we report the cloning of *RCR1* (*Resistance to Clubroot 1*) and show that *RCR1* is a *P. brassicae*-induced resistance gene in *Arabidopsis thaliana*. RCR1 is localized the endoplasmic reticulum (ER). Overexpression of *RCR1* induces programmed cell death in *Nicotiana benthamiana*. In addition, we show that RCR1 forms an oligomer when expressed in *Arabidopsis* protoplasts. We propose that RCR1 is an inducible protein that triggers programmed cell death by an unknown mechanism. Next, we will try to elucidate its function and explore its potential to improve disease resistance in crop plants.

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* Avr 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.

An atlas of plant NLR proteins

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In order to assess the spread and diversity of plant NLRs, we compiled a database assembling all transcripts from UniprotKB found to comprise both NBS and at least one CC/TIR/LRR domain using code we recently developed. We identified so far ~70k proteins matching these requirements that were further analyzed with respect to their domain organization, motif delineation and structural properties profiles. Sequences showing regular NLR organization were grouped according to their taxonomic distribution and clustered to thresholds from 30–90%. The first version of this interconnected database is available on web along with several tools for data exploration, visualization and analysis which allow users to zoom-in on particular NLR groups and operate detailed bioinformatic analyses such as interactive MSA synchronized with secondary structure and LRR motif predictions, cluster identity matrices and variability analysis of local features useful in sequence to 3D mapping. Tools are also under development for hotspot identification and the investigation of correlated mutations related to inter-domain/protein interactions. The atlas under development around these core concepts is intended to deliver an overall structured view of plant NLRs along with a large collection of easy, ready to use bioinformatic tools tailored for NLR structure informed functional research and the exploration of the complex sequence landscape surrounding any particular NLR.

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.

Investigating Avr3 effector function and activation of signal transduction and defence responses following recognition by I-3

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The fungus *Fusarium oxysporum* causes devastating wilt diseases in plants. The interaction between tomato and *F. oxysporum* f. sp. *lycopersici* (*Fol*) is an important model system for the study of plant/*F. oxysporum* interactions. Tomato has four immunity genes (*I*, *I-2*, *I-3*, and *I-7*) that enable it to recognize and respond to *Fol* isolates carrying the corresponding avirulence genes (*Avr1*, *Avr2*, *Avr3*, and *Avr7*). To study the virulence function of *Avr3* and its activation of signaling and defence components following recognition by *I-3*, *Avr3* was expressed in *E. coli* and purified for experimental use. Purified *Avr3* protein was infiltrated into *Nicotiana benthamiana* leaves to look for a possible virulence function. *Avr3* was found to induce callose deposition and suppress flg22-induced ROS accumulation in *N. benthamiana*. In tomato, infiltration of *Avr3* protein into leaves was found to induce an *I-3*-dependent cell-death response indicative of defence activation. This response has been used along with Virus Induced Gene Silencing (VIGS) to develop an assay to study downstream signaling components involved in the *I-3* response. Unlike most other resistance genes, *I-3* encodes an S-receptor-like kinase, so this study will help us learn more about a novel plant defence-activation pathway.

Engineering Sr33 binding specificity to alter effector recognition

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Plants have evolved cytoplasmic immune receptors called NLRs that recognize the presence of a pathogen through a range of mechanisms: direct binding of effectors or indirect recognition of effector actions. The direct binding of effectors has been shown to be mediated through the NLR's Leucine Rich Repeat (LRR) domain. Accurate prediction of amino acids involved in these direct interactions can greatly enhance understanding of effector recognition and inform efforts to engineer new resistance. In this study, we utilized two NLR resistance genes from wheat, Sr33 and Sr50, that recognize distinct effectors by directly binding to them through their LRR. While the effector recognized by Sr50 is known and described as AvrSr50, the effector recognized by Sr33 remains unknown. Through a combination of phylogenomics, allele diversity analysis in the LRR and structural modeling we identified the amino-acids in Sr50 likely to physically interact with its effector. Mutation of these sites helped identify 12 amino acids we hypothesized to be sufficient to mediate effector binding in Sr50. Furthermore, we were able to pinpoint amino acid residues that govern auto-activity of Sr50 in the wheat protoplast cell death assay. When changing these 12 corresponding amino acids in Sr33, we see AvrSr50-dependent initiation of cell death in *Nicotiana benthamiana*. These findings are a major advance towards the successful engineering of new effector recognition specificities in direct binder NLRs.

Characterization of E3 Ubiquitin Ligase 1 as a novel regulator of PTI-associated resistance in *Arabidopsis thaliana*

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E3 Ubiquitin Ligase 1 (E3L1), a plasma membrane-localized E3 Ligase in *Arabidopsis thaliana*, has previously been shown as being rapidly phosphorylated during pattern-triggered immunity (PTI)-associated defense responses. In this work, our characterization of T-DNA insertional mutants (e3l1-1, e3l1-2) implicate E3L1 as a positive regulator of PTI. The mutant plants are compromised in their ability to manifest PTI-induced resistance following pretreat with flg22. However, several other typical defense related phenotypes, including defense transcript accumulation, ROS production, and ligand-induced endocytosis of FLS2, were unaltered, indicating that E3L1 functions downstream or independently of these responses. In a previous study, we demonstrated that loss of MAP Kinase Phosphatase 1 (MKP1) results in increased resistance against *Pseudomonas syringae* pv. *tomato* DC3000 (DC3000). This resistance was shown to be caused by a decrease in secretion of extracellular T3SS-inducing metabolites, indicating alterations in metabolite transporter activity or accumulation in mkp1. Due to the predicted function of E3L1 targeting proteins for degradation or endocytosis at the plasma membrane, we hypothesized a genetic link between E3L1 and MKP1. Infection of a double mutant, e3l1 mkp1, shows a suppression of mkp1-related resistance, genetically placing E3L1 in the MKP1-dependent pathway.

***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.

Disruption of barley immunity to powdery mildew by an in-frame Lys-Leu deletion in the essential protein SGT1

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Barley *Mla* (*Mildew resistance locus a*) and its nucleotide-binding, leucine-rich-repeat receptor (NLR) orthologs protect cereal grains from diseases caused by fungal pathogens. Yet, many intricacies of MLA protein-protein interactions and their effect on MLA-associated recognition remain unknown. To further characterize the molecular interactions required for NLR-based immunity, we used fast-neutron mutagenesis to screen 34,800 M2 families for plants compromised in MLA-mediated response to the powdery mildew fungus, *Blumeria graminis* f. sp. *hordei*. One variant, m11526, contained a novel mutation, designated *rar3* (*required for Mla6 resistance3*), that abolishes race-specific resistance conditioned by the *Mla6*, *Mla7*, and *Mla12* alleles, but does not compromise immunity mediated by *Mla1*, *Mla9*, *Mla10*, and *Mla13*. We used bulked-segregant-exome capture and fine mapping to delineate the causal mutation to an *in-frame* Lys-Leu deletion within the SGS domain of SGT1 (Suppressor of G-two allele of *Skp1*, *Sgt1*_{ΔKL308-309}), the structural region that interacts with MLA proteins. In nature, mutations to *Sgt1* are usually lethal, but we pinpoint a unique modification that delineates its requirement for some disease resistances, while unaffected others as well as normal cell processes. Further study could distinguish the regions by which pathogen effectors and host proteins interact with SGT1, facilitating precise editing of effector-incompatible variants. DOI: <https://doi.org/10.1093/genetics/iyaa026>.

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.

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.

A GH-BBEL-POD machinery converts a cell wall damage into a controlled oxidative signal

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The recent identification of several plant berberine bridge enzyme-like (BBELs) proteins as oxidoreductases of specific cell wall fragments that act as damage-associated molecular patterns (DAMPs) opened the way to novel insights in plant immunity. The oxidation of cell wall DAMPs by BBEL enzymes quenches their elicitor activity by concomitantly releasing H₂O₂. Little is known about the role of oxidized oligomers and of the H₂O₂ released from their oxidation. By a multiple-enzyme assay approach, we provide an in vitro demonstration that the H₂O₂ generated by the combined action of a microbial glycosyl hydrolase (GH) such as a polygalacturonase or a cellulase and a plant BBEL can convert the hydrolysis of a cell wall polysaccharide into an oxidative signal. The substrate specificity of the GH-BBEL couple can be used to modulate the signaling outcome. Moreover, if a plant peroxidase (POD) is added to such reaction, H₂O₂-mediated oxidation can be successfully extended to crucial processes such as monolignol and IAA oxidation, other events that are strictly related to plant defence. Differently from an oxidative burst, the oxidizing potential of the H₂O₂ generated by BBELs is modulated by the antioxidant capability of the same oxidized oligomer from which it originates, demonstrating that H₂O₂ and oxidized oligomers both contribute to convert a microbial GH activity into a controlled oxidation signal, useful for fine-tuning and modulating appropriate defence responses over time.

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.

Role of nuclear targeting effectors of Brassicaceae smut fungus *Thecaphora thlaspeos* in modulation of host transcription

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Thecaphora thlaspeos rises as a novel infection system of *Brassicaceae*, specifically infecting *Arabidopsis* species and *A. thaliana*. It establishes a long-lasting biotrophic interaction with host plants without causing any macroscopic symptoms. Pathogens depend on secreted effector proteins for establishing biotrophic interaction. My project deals with the characterization of nuclear localized (NLS) effectors of *T. thlaspeos*. Transcriptome data of infected plants revealed that 132 fungal genes are induced including 40 effector candidates. Two candidates, Tuel0 and Tael2 have a significant effect on bacterial proliferation in a heterologous system. Transient expression of Tuel0 and Tael2 have shown their accumulation in both nucleus and nucleolus. However, NLS deletion shows exclusion of the signal from the nucleolus. Besides that, *A. thaliana* expressing Tuel1 has a dwarf phenotype, virulence activity and nuclear localization. Transcriptome analysis of Tuel1 line shows altered gene expression with 93 up-regulated and 12 down-regulated genes. There is a prediction of transcription factor binding sites in differentially regulated genes of Tuel1 line and DNA binding residues in Tuel1, Tuel0, and Tael2, so these predictions and verification of NLS strengthen our hypothesis that these candidates might act as a transcription factor. Further analysis of DNA binding activity via EMSA is in progress. This data might assist in studying the interference of NLS effectors with plant immune responses.

The MIK2 receptor senses SCOOP phytochemicals and microbial proteins and triggers immunity

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Sessile plants encode a large number of small peptides and cell surface-resident receptor kinases, most of which have unknown functions. MALE DISCOVERER 1-INTERACTING RECEPTOR-LIKE KINASE 2 (MIK2) has been implicated in pollen tube growth and plant disease resistance. Here, we present data to show that MIK2 recognizes plant endogenous SERINE RICH ENDOGENOUS PEPTIDE (SCOOP) family peptides and elicits immune responses. SCOOP signature peptides trigger diverse immune and physiological responses in a MIK2-dependent manner on a subnanomole scale. SCOOPs directly bind to the extracellular leucine-rich repeat domain of MIK2 in vivo and in vitro, indicating that MIK2 is the receptor of SCOOP peptides. Perception of SCOOP peptides induces the association of MIK2 and the coreceptors SOMATIC EMBRYOGENESIS RECEPTOR KINASE 3 (SERK3) and SERK4 and relays the signaling through BOTRYTIS-INDUCED KINASE 1 (BIK1) family cytosolic receptor-like kinases. Surprisingly, SCOOP-LIKE (SCOOP-L) signature motifs are also present in the genomes of a wide range of fungal *Fusarium* spp. and bacterial *Comamonadaceae*. *Fusarium* and *Comamonadaceae* SCOOP-L peptides activate MIK2-BAK1/SERK4 dependent immune responses, pointing to a dual role of MIK2 in the perception of both microbe- and damage-associated molecular patterns (MAMPs/DAMPs).

Resistance of *Arabidopsis thaliana* against *Botrytis cinerea* induced by biological elicitors involves extensive priming of transcriptional responses to pathogen infection

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Plants have evolved an innate immune system based on the recognition of pathogen- and host-derived elicitors by specific pattern recognition receptors. Exogenous treatments with elicitors often lead to an increased resistance against phytopathogenic microorganisms, which is maintained for several days after treatment. This induced resistance can be partly explained by the phenomenon of priming, which leads to a faster and stronger response against subsequent attacks. The exact mechanisms at the basis of this long-lasting resistance induced by elicitors is however poorly understood. We have found that pretreatments of *Arabidopsis* plants with flg22 confer resistance to the pathogen *Botrytis cinerea* that lasts for at least one week without a reduction of fitness. We performed a whole genome-scale analysis of the transcriptome of *Arabidopsis* plants treated with flg22 and subsequently inoculated with *B. cinerea* to identify genes regulated by long-term treatments with flg22 or primed during infection in pre-treated plants. This analysis revealed that plants pre-treated with flg22 show extensive changes in gene expression during pathogen infection, compared to water-treated plants. The obtained data could help clarify the mechanism of elicitor-induced resistance and identify potential targets to improve crop resistance with minimal fitness cost.

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.

Four candidate avirulence effector loci contribute to *Pseudomonas syringae* pv. *actinidiae* biovar 3 recognition in *Actinidia arguta*

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Pseudomonas syringae pv. *actinidiae* (Psa), the causal agent of kiwifruit canker, is a recently emerged plant pathogen. A pandemic isolate of Psa biovar 3 (Psa3) has devastated kiwifruit orchards growing cultivars of *Actinidia chinensis*. In contrast, *A. arguta* (kiwiberry) is resistant to Psa3. While previous research has identified Psa3 effectors required for virulence, no specific Psa3 avirulence effectors recognized by the host have been identified in *Actinidia*. Screening of a complete library of Psa3 effector knockout strains identified four effectors—avrRpm1, HopF2, HopZ5, and the exchangeable effector locus effector HopAW1—associated with the resistance response in *A. arguta*. These results were corroborated by biolistic bombardment in a reporter eclipse assay. In turn, these avirulence effectors can be used to identify the first cognate resistance genes in *Actinidia* for breeding durable resistance into future kiwifruit cultivars.

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.

Synthesis and evaluation of green nanoparticles against *Colletotrichum truncatum* inciting anthracnose of soybean in India

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This is the first report on assessing the effectiveness of four green synthesized nanoformulations viz., chitosan based zinc nanoformulation (ChZnNF), *P. fluorescens* based zinc nanoformulation (PfZnNF), pomegranate aril based sulfur (PASNF) and silver nanoformulation (PAAgNF) at different concentration under in vitro and glasshouse conditions. Characterization by PSA and SEM revealed particle size range 9.9 to 83.5 nm and circular to irregular. Elemental confirmation was performed by EDS. PAAgNF was effective in suppressing *C. truncatum* by inhibition up to 82.75% under laboratory. PAAgNF was effective at 500 ppm reducing PDI up to 100 and 96.97% in JS 335 and DSB 21 variety which is on par with recommended fungicide. LC-MS analysis of PAAgNF revealed major role of phenolic acids (hydroxy benzoic acid, protocatechuic acid, gallic acid, caffeic acid, and ferulic acid) and flavonoids (naringenin and epicatechin) in antimicrobial activity. Green synthesized PAAgNF have can be explored as a tool in managing *Colletotrichum truncatum*.

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.

UDP-dependent glycosyltransferase 76B1 is critical to fine-tune plant immunity via N-hydroxypipicolinic acid glycosylation

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Salicylic acid (SA) and N-hydroxypipicolinic acid (NHP) are two key signaling molecules that accumulate in the model plant *Arabidopsis thaliana* in response to biotrophic pathogens. One mechanism to control their cellular concentration is the conversion to sugar-derivatives via UDP-dependent glycosyltransferases (UGTs). In contrast to SA metabolizing UGTs, enzymes using NHP as substrate were not described so far. We investigated plant immune metabolism via non-targeted UHPLC-HRMS of a promising knockout-mutant for *ugt76b1*, which lead us to identify NHP as UGT76B1s substrate. We underlined the observation by quantitative MS analysis of t-DNA and CRISPR/Cas9 knockout mutants, as well as in vitro UGT76B1 assays and tracking studies with D₉-NHP. Via tandem-MS-analyses, we were able to show that UGT76B1 is catalyzing the formation of NHP-OGlc in vitro and in vivo. Independent *ugt76b1* mutant alleles exhibited an enhanced resistance phenotype towards *Hyaloperonospora arabidopsidis* Noco2 and virulent *Pseudomonas syringae* ES4326 strain as well as dwarfism compared to wild type. As the *fmo1 ugt76b1* double mutants do not exhibit growth deficiency and enhanced resistance, we conclude that UGT76B1 acts downstream of FMO1 and is critical to balance growth and defense by modulating the NHP concentration. These results underline the central role of UGT76B1 on metabolic turnover in plant defense metabolism.

Dynamic ubiquitylation and deubiquitylation in plant pattern-triggered immunity

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Protein ubiquitylation profoundly expands proteome functionality and diversifies cellular signaling processes. Ubiquitination is a reversible post-translational modification process mediated by Ubiquitin (Ub) ligases (E3s) and deubiquitinases (DUBs), with recent studies providing ample evidence for its importance to plant immunity. To gain a proteome-wide appreciation of ubiquitylome dynamics during immune recognition, we employed a two-step affinity enrichment coupled with high sensitivity mass spectrometry to identify *Arabidopsis* proteins rapidly ubiquitylated upon plant perception of the microbe-associated molecular pattern (MAMP) peptide flg22. The catalog from *Arabidopsis* treated for only 30 min with flg22 contained nearly 1,000 conjugates, 150 Ub footprints, and all seven types of Ub linkages, and included previously uncharacterized conjugates of immune components. In vivo ubiquitylation assays confirmed modification of several candidates upon immune elicitation and revealed distinct modification patterns and dynamics. Gene ontology and network analyses of the collection also uncovered rapid modification of the Ub-proteasome system itself, suggesting a critical auto-regulatory loop necessary for an effective MAMP-triggered immune response and subsequent disease resistance. Collectively, the proteomic analyses further strengthened the connection between ubiquitylation and flg22-based immune signaling, and increased the database of ubiquitylated substrates in plants.

Characterizing a TIR-only/CC-NBS-LRR dual receptor system for plant pathogen recognition

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RBA1 is an Arabidopsis TIR-only immune receptor responsible for the recognition of the *Pseudomonas syringae* type III effector, HopBA1. RBA1 is unusual, lacking C-terminal domains that regulate canonical TIR-NBS-LRR (TNL) immune receptors. In the Arabidopsis accession Ag-0, recognition of HopBA1 by RBA1 triggers cell death. In other Arabidopsis accessions (e.g., Col-0), HopBA1 is instead recognized by ZAR1, a CC-NBS-NLR (CNL), to trigger disease resistance without cell death. It is currently unclear if RBA1 and ZAR1 immune responses are independent or if one regulates the other. To determine the genetic requirements for cell death and disease resistance in Ag-0 we have generated *rba1*, *zar1*, and *rba1/zar1* Cas9 mutants. These mutants reveal the hypersensitive response to HopBA1 in Ag-0 previously attributed to RBA1 also requires ZAR1. ZAR1 is known to be activated upon recognition of modified PBL kinases. We hypothesize that HopBA1 targets one or more PBL kinases, leading to both ZAR1 and RBA1 activation. To identify putative HopBA1 virulence targets, we have screened the Arabidopsis PBL kinase protein family for physical interactions with HopBA1. The biological relevance of these interactions is being assessed using reverse genetics in Arabidopsis. The HopBA1/RBA1/ZAR1 system will help explain how structurally diverse receptors can work together to form an immune system that appropriately responds to pathogens to trigger disease resistance and, in some cases, cell death.

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>.

Translating -omics big data: Comprehensive understanding of host-pathogen interactions to control bacterial blight in alfalfa using computational approaches

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Molecular interactions between host and pathogen forms the basis of pathogenesis. Majority of *Pseudomonas syringae* strains are known to cause frost injury in plants, amongst which, *P. syringae* pv. *syringae* ALF3 is asserted to be the causal organism of bacterial stem blight in *Medicago sativa* (alfalfa). We elucidated genome-scale host-pathogen interactions (HPIs) between alfalfa and *P. syringae* using two intense computational approaches: interolog and domain-based method, followed by functional enrichment, subcellular localization, and network visualization of the proteins. In total, ~14M probable HPIs were predicted from both the approaches, involving 50,629 alfalfa and 2,932 *P. syringae* proteins. The functional enrichment analysis revealed that alfalfa proteins are involved in cellular to chemical stimulus (GO:0070887), oxidoreductase activity (GO:0016614) and chloroplast stroma (GO:0009570), which is considered as a major production house of various defense-related signals (ROS) and hormones (abscisic acid, jasmonic acid). Alfalfa proteins are also found to be enriched in plant-pathogen interaction pathway (mtr04626), whereby multiple immune responses are generated against invading pathogens. The present study is the first to report advanced computational approaches for deciphering genome-scale host-pathogen PPIs between alfalfa and *P. syringae*, thus enabling the researchers to better understand the infection mechanisms of the pathogen and develop pathogen-resistant lines.

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.