Macroinfluence of Microorganisms: Host-Microbe Interactions and Inspired Technologies

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Poster Abstracts
The important of symbiotic microbes in termite lignocellulose digestion

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Lower termites are important models for insect-microbe symbiosis because of the diversity, complexity and functionality of their unique tripartite symbiosis. This collaboration consisting of all three domains of life allows termites to thrive on a diet of nitrogen-poor lignocellulose. The eastern subterranean termite, Reticulitermes flavipes, houses over 4,000 species of protists, bacteria and archaea living symbiotically in its gut. Recent investigations of lignocellulose digestion in R. flavipes and other lower termites have primarily focused on the functional contributions of the eukaryotic members of the termite holobiont (termite and protist). Here, using multiple antimicrobial treatments, differing degrees of dysbiosis were induced in the termite gut, leading to variably altered prokaryotic abundance, prokaryotic diversity and lignocellulolytic capacity. These findings quantify the saccharolytic role, directly or indirectly, of prokaryotic members within the termite gut holobiome. This is specifically manifested by reductions of 23-47% and 30-52% in glucose and xylose yields respectively from complex lignocellulose. Thus, all members of the lower termite holobiont (termite, protists and prokaryotes) collaborate for efficient, sustained lignocellulase activity. This unprecedented quantification emphasizes of the relevance of lower termites, like R. flavipes, as models for inter-domain symbioses.
Direct regulation of plant Argonautes by microRNA-targeted transcription factors.

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Argonautes are the central effector proteins of RNA silencing which bind target transcripts in a small RNA-guided manner. Arabidopsis has ten Argonate proteins, with specialized roles in RNA-directed DNA methylation, post-transcriptional gene silencing, and anti-viral defense. To better understand specialization among Argonautes (AGO) at the level of transcriptional regulation we tested a library of 1541 transcription factors for binding to the promoters of AGO1, AGO10, and AGO7 using yeast 1-hybrid assays. A ranked list of candidate DNA-binding TFs revealed binding of the AGO7 promoter by a number of proteins in the TCP and SPL families, which are known to be involved in developmental timing and leaf morphology. We are testing the functional significance of these binding sites for AGO7’s role in vegetative phase change and its polar expression pattern. Reverse genetic, transgenic, ChIP, and expression profiling approaches will be used to define the regulatory network involving AGO7, SPLs, TCPs, and the microRNAs that regulate them.
**Structural Basis for Regulation of Rhizobial Nodulation and Symbiosis Gene Expression by NolR**

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The symbiosis between rhizobial microbes and host plants involves the coordinated expression of multiple genes, which leads to nodule formation and nitrogen fixation. As part of the transcriptional machinery for nodulation and symbiosis across a range of Rhizobium, NolR serves as a global regulatory protein. Here we present the x-ray crystal structures of NolR in the unliganded form and complexed with two different 22 base pair (bp) double-stranded operator sequences (oligos AT and AA). Structural and biochemical analysis of NolR reveals protein-DNA interactions with an asymmetric operator site and defines a mechanism for conformational switching of a key residue (Gln56) to accommodate variation in target DNA sequences from diverse rhizobial genes for nodulation and symbiosis. This conformational switching alters the energetic contributions to DNA binding without changes in affinity for the target sequence. Two possible models for the role of NolR in the regulation of different nodulation and symbiosis genes are proposed. These studies provide the first structural insight on the regulation of genes involved in the agriculturally and ecologically important symbiosis of microbes and plants that leads to nodule formation and nitrogen fixation.
Screening natural variation in the genus *Camelina* using high-throughput phenotyping and genotyping

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The projected growth of human population and changing demographics over the next century are expected to increase demand for food and plant products, but environmental considerations require that production increases are accomplished while using less water, land and chemical inputs per unit of yield. One strategy to increase productivity with minimal impact on existing food production is the development of new crops and cultivars that are well suited for industrial uses, such as fuel production, that are productive on suboptimal land with limited irrigation. *Camelina sativa* is an oilseed crop from the family *Brassicaceae* that is an emerging source of oil for fuel. *C. sativa* grows well with few inputs and can be grown as a rotation crop with conventional summer annual crops. Our overall goal is to identify traits associated with yield and water utilization in *C. sativa* wild germplasm to enhance breeding efforts. We utilize robotic, image-based plant phenotyping to rapidly and quantitatively measure phenotypic diversity among *C. sativa* natural accessions using the Bellwether Foundation Phenotyping Facility at the Donald Danforth Plant Science Center. The Danforth Center phenotyping system combines a controlled-environment Conviron growth house with a LemnaTec Scanalyzer 3D conveyor and imaging system that is capable of collecting phenotypic data for 1140 plants for several weeks per experiment. Data is collected from watering and weighing stations and cameras for visible spectrum color imaging for measuring biomass, growth and shape-based traits; near-infrared reflectance imaging for measuring relative tissue water content; and chlorophyll florescence imaging for measuring photosynthetic efficiency. Image processing and data analysis are done using custom open-source software. In future work we will identify markers associated with phenotyped traits using genome-wide association mapping.
Fostering Plant Science Research at MU Plant Transformation Core Facility

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University of Missouri (MU) Plant Transformation Core Facility has been providing state-of-the-art plant transformation services over the past 14 years. The facility is aiming at fostering plant science research by providing transformation services worldwide. The services are on fees for cost recovery only, not for profit. The facility staff is dedicated to providing various types of transformation services with a focus on maize (Zea mays), soybean (Glycine max), switchgrass (Panicum virgatum), sorghum (Sorghum bicolor), wheat (Triticum aestivum), alfalfa (Medicago truncatula), as well as Setaria viridis. The service categories include both standard and customized transformation. Transformation systems for all crops utilize Agrobacterium-mediated approaches and somatic embryogenesis processes except for soybean and Medicago. The Agrobacterium-mediated cot-node transformation system coupled with organogenesis regime is employed for soybean and Medicago transformation. The facility is also ready to take on new service projects to transform new plant species as user’s requests. Research activities are geared towards developing high-throughput transformation systems, effective small RNA-mediated gene silencing, gene stacking through coordinated transgene expression, and precise genome modifications to meet the needs of crop improvement and genome discoveries. More details on the facility can be found at http://www.plantsci.missouri.edu/muptcf.
Heat shock protein 90.1 plays a role in Agrobacterium-mediated plant transformation

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Heat shock protein 90 (HSP90) is a molecular chaperone responsible for the maturation and stability of a large number of proteins. Transient and stable root transformation assays of Arabidopsis HSP90.1 knock-out mutants and overexpression lines revealed that the severity of plant susceptibility to Agrobacterium-mediated transformation correlated with the level of HSP90.1 expression. Arabidopsis thaliana HSP90.1 interacts with Arabidopsis VirE2 Interacting Protein 1 (VIP1). The interaction between HSP90.1 and VIP1 was demonstrated through Bimolecular Fluorescence Complementation (BiFC), yeast two-hybrid, and in vivo pull-down assays. The HSP90 inhibitor geldanamycin (GDA) caused aggregation of VIP1 in Nicotiana benthamiana leaves. Following infiltration of Agrobacterium tumefaciens GV3101 harboring a binary vector encoding YFP-VirE2 into tobacco leaves, VirE2 showed an intense cytoplasmic signal, consistent with aggregation, after GDA treatment. We further demonstrated that induction of the defense gene PR1 was diminished in a hsp90.1-2 mutant after flg22 application. We propose that HSP90.1 functions as a VIP1 molecular chaperone, and contributes to Agrobacterium-mediated plant transformation.
Fighting ROS and Aging Related Diseases: A Step Toward Uncoupling Socioeconomic Status and Diet Quality

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Reactive oxygen species (ROS) are associated with a number of aging related diseases in humans, such as cancer, Alzheimer’s Disease, and cardiovascular disease. Research suggests that diets rich in phenolic antioxidants may help prevent the onset of these diseases by preventing oxidation damage caused by ROS. However, a multitude of cross-sectional studies conducted in developed countries, including the United States, showed that consumption of fruits and vegetables traditionally recognized for containing high levels of phenolic antioxidants follows a socioeconomic gradient. Low socioeconomic status groups, defined by lack of education and income, tend to consume cheap, highly processed, and energy-dense foods rather than fruits and vegetables containing phenolic antioxidants. However, grains, especially maize, are known to possess high amounts of phenolic antioxidants. Furthermore, grains are key ingredients in processed snack foods and ready-to-eat cereals. As a first step toward improving the phenolic antioxidant content of maize-endosperm based foods through breeding, the variation in type and quantity of phenolic antioxidants such as ferulic acid, p-coumaric acid, and sinapic acid were examined in elite U.S. maize germplasm typical of that used in the production of maize-endosperm based foods.
Loss of the Arabidopsis thaliana Dynamin-Related Protein 2B Reveals Separation of Innate Immune Signaling Pathways

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Vesicular trafficking is a critical means by which eukaryotes modulate responses to microbial pathogens. However, considering the complexity of membrane trafficking in plants, relatively few trafficking components with functions in plant immunity are known. Here, we demonstrate that Dynamin-Related Protein 2B (DRP2B), previously implicated in constitutive clathrin-mediated endocytosis (CME), functions in responses to bacterial flagellin and immunity against Pto DC3000. Analysis of Arabidopsis thaliana drp2b null mutants revealed three distinct branches of the flagellin signaling network that differ in their requirement for RbohD, the NADPH oxidase responsible for flagellin-induced apoplastic reactive oxygen species production. Dissection of these DRP2B-regulated responses provided insights into how diverse flagellin-signaling branches are integrated for effective immune-signaling and resistance to bacteria. Based on live-cell imaging studies, flagellin-elicited internalization of the plant flagellin-receptor, FLAGELLIN SENSING 2 (FLS2), was found to be partially dependent on DRP2B, thus providing evidence for CME components in ligand-induced endocytosis of FLS2. Reduced trafficking of FLS2 in response to flagellin may contribute in part to the non-canonical combination of immune signaling defects observed in drp2b. In conclusion, DRP2B aids to integrate the modulation of FLS2 trafficking and distinct flagellin-signaling branches, highlighting the importance of a functional vesicular trafficking system in plant innate immunity.
Type III effectors and host specificity in Xanthomonas pathogens

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Cassava is a key staple crop that feeds over 800 million people worldwide. Among the disease threats to this crop is cassava bacterial blight, which is caused by the pathogen Xanthomonas axonopodis pv. manihotis (Xam). Similar to other bacterial pathogens, Xam uses a type III secretion system to secrete an array of virulence factors, known as type III effectors (TTEs), into host cells. TTEs are collectively necessary for Xam to colonize host tissue and cause disease. The effects of individual TTEs on virulence are currently under investigation. Most bacterial plant pathogens are able to infect only a small number of plant species. One hypothesis to explain this observation is that the TTE repertoire of a given strain determines its host specificity. To investigate this hypothesis, a comparative genomics study was conducted between Xam and several closely related pathogens that infect castor oil plant and poinsettia, which are members of the Euphorbiaceae family along with cassava. Draft genome sequences for these strains were generated, and TTE repertoires were predicted based on these sequences. The virulence levels of these strains relative to Xam are currently being assessed. These data will enable hypotheses to be generated regarding specific TTEs that determine host specificity.
Deciphering the roles of host innate immunity in shaping the structure and assembly cues of leaf surface microbiota.

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The innate immune system of plants protects them from pathogens while allowing colonization by the commensal and symbiotic microbes. A clear understanding of the roles plant defense signaling pathways and the genes encoding antimicrobial peptides play in shaping the structure and assembly of plant microbiota is currently lacking. We are characterizing the leaf and stem microbiomes of the wild-type Arabidopsis thaliana (Col-0), mutants defective in salicylic acid and jasmonic acid signaling pathways and transgenic lines over-expressing antifungal defensins. For this study, we selected four natural soils with varying profiles from Oklahoma and Missouri. Soil pH, organic matter, organic and inorganic P-N-K composition and the ability to sustain both the wild type and mutant Arabidopsis lines were taken into consideration. MiSeq paired-end sequencing of 16S rDNA V4 region will be performed to characterize baseline microbial communities. Arabidopsis wild type and mutants will be transplanted so that leaf and stem samples can be collected at different developmental stages of plants. Sequence analyses will be performed using Quantitative Insights into Microbial Ecology (QIIME). We expect to derive microbial assembly patterns that shed light on how plants recruit symbiotic microbes, and modulate host innate immune response shaping leaf surface assembly cues. Funding acknowledgement: Association of Independent Plant Research Institutes (AIPI)
Non-Homologous End-Joining Proteins Limit *Agrobacterium* T-DNA Integration Into the Plant Genome.

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Non-homologous end-joining (NHEJ) is the major model proposed for *Agrobacterium* T-DNA integration into the plant genome. Several proteins, including KU70, KU80, ARTEMIS, DNA-PKcs, DNA Ligase IV (LIG4), ATM, and ATR, play an important role in “classical” (c)NHEJ. Other proteins, including histone H1 (HON1), XRCC1, and PARP1, participate in a “backup” (b)NHEJ process. We examined transient and stable transformation frequencies of *Arabidopsis* roots mutant for these and other genes. Mutants in *KU70*, *KU80*, and the plant-specific DNA LIGASE VI (LIG6) showed increased stable transformation susceptibility. However, these mutants showed transient transformation susceptibility similar to that of wild-type plants, suggesting enhanced T-DNA integration in these mutants. These results were confirmed using a promoter-trap transformation vector that requires T-DNA integration into the plant genome to activate a promoterless *gusA* gene, by virus-induced gene silencing (VIGS) of *Nicotiana benthamiana* NHEJ genes, and by biochemical assays for T-DNA integration. No alteration in transient or stable transformation frequencies was detected with *atm*, *atr*, *lig4*, *hon1*, *xrcc1*, or *parp1* mutants. However, mutation of *parp1* caused high levels of T-DNA integration and transgene methylation. We hypothesize that plants mutant in several NHEJ genes show increased T-DNA integration because delayed dsDNA break-repair provides greater opportunity for T-DNA integration.
Is VIP1 important for *Agrobacterium*-mediated transformation?

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*Agrobacterium* genetically transforms plants by transferring and integrating T-(transferred) DNA into the host genome. This process requires both *Agrobacterium* and host proteins. VIP1 (VirE2 interacting protein 1), an *Arabidopsis* bZIP protein, has been suggested to mediate transformation through interaction with and targeting of VirE2 to nuclei. Various *Agrobacterium* strains were used to examine the transformation susceptibility of *Arabidopsis vip1* mutant and VIP1 overexpressing plants. No altered transformation susceptibility was detected. Confocal microscopy was used to examine the subcellular localization of Venus-tagged VirE2 or Venus-tagged VIP1, in the presence or absence of the other untagged protein, in different plant cell systems. VIP1-Venus localized in both the cytoplasm and the nucleus of *Arabidopsis* roots, agroinfiltrated *Nicotiana benthamiana* leaves, *Arabidopsis* mesophyll protoplasts, and tobacco BY-2 protoplasts, regardless of whether VirE2 was co-expressed. VirE2 localized exclusively to the cytoplasm of tobacco and *Arabidopsis* protoplasts, whether in the absence or presence of VIP1 overexpression. In transgenic *Arabidopsis* plants and agroinfiltrated *N. benthamiana* leaves, small aggregates of Venus signal occasionally appeared in nuclei, but these were likely imagining artifacts. The vast majority of VirE2 remained in the cytoplasm. These results indicate that VIP1 is not important for *Agrobacterium*-mediated transformation or VirE2 subcellular localization.
THE ARABIDOPSIS IMMUNE ADAPTOR SRFR1 INTERACTS WITH TCP TRANSCRIPTION FACTORS THAT REDUNDANTLY CONTRIBUTE TO EFFECOR-TRIGGERED IMMUNITY

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Effector-triggered immunity needs to be tightly controlled both positively and negatively to enable normal plant growth, because constitutively activated defense responses are detrimental to the host. Mutations in SRFR1 (SUPPRESSOR of rps4-RLD1), identified in a suppressor screen, reactivated avrRps4-triggered immunity. Resistance in srfr1 mutants was not effective against virulent pathogens, suggesting that SRFR1 is a specific negative regulator of effector-triggered immunity. To date, the function of SRFR1 appears to be limited to resistance proteins of the TIR-NB-LRR class that require functional EDS1 to trigger immunity. SRFR1 encodes a pioneer tetratricopeptide repeat (TPR) protein conserved between plants and animals. The SRFR1 TPR domain has significant sequence similarity to those of the Saccharomyces cerevisiae Ssn6 and Caenorhabditis elegans OGT (O-linked N-acetylglucosamine transferase) proteins, which function as transcriptional repressors. Here we show that SRFR1 physically interacts with members of the TEOSINTE BRANCHED1/CYCLOIDEA/PCF (TCP) transcription factor family. In addition, higher-order tcp knock-out plants were compromised in effector-triggered immunity, and constitutive PR2 expression in srfr1-4 plants was attenuated by mutations in SRFR1-interacting TCPs. Given that TCP transcription factors to date have largely been shown to regulate developmental processes, we propose that nuclear SRFR1 functions in a transcriptional repressor complex that balances plant immunity and development.
Transcriptomic response of flax (*Linum usitatissimum* L.) to infection by *Fusarium oxysporum* lini.

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Canada is a major exporter of flax, which is a source of valuable bioproducts derived from the seed and the stem fiber. Fusarium wilt has been a limiting factor in the production of flax worldwide. In the present study, the infection of *Fusarium oxysporum* in flax over a three week period was followed. Disease symptoms were evident both externally and in anatomical root preparations. Selected chitinase genes, which are critical in the response of plants to pathogens, were overexpressed in treated flax plants. RNAseq analyses showed enriched functional categories related to plant defense with concomitant increase in the number of differentially expressed genes over time. The increased number of overexpressed and repressed genes through the time course established the relevance of key metabolic pathways including: biosynthesis of phenylpropanoids, terpenoid biosynthesis, plant pathogen interaction, plant hormone signal and carbon metabolism.
RNAi machinery in *Colletotrichum higginsianum* has a role in antiviral defense and plant pathogenesis.

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Members of *Colletotrichum* cause disease in over 3000 plant species, including most crops. *C. higginsianum* infects *Arabidopsis* providing the opportunity to study and manipulate both host and pathogen. To characterize the role of RNAi in *C. higginsianum* vegetative and plant-pathogen interactions, knock-out mutants of the RNAi machinery genes were created. mRNA and small RNA from mycelial tissue from each mutant were sequenced to identify small RNA-producing loci. As well, immunoprecipitation of AGO1 protein followed by small RNA sequencing was used to specifically identify small RNAs loaded into AGO1. The greatest effect on RNA populations was in the *dcl1* and *ago1* strains related to the de-repression of an uncharacterized dsRNA virus. Viral infections of fungi are primarily asymptomatic. In *C. higginsianum* no effect was observed on vegetative growth of any mutant when grown on complex or minimal media. However, *dcl1* and *ago1* strains produced six- and three-fold fewer conidia, respectively. As the initial biotrophic phase of infection by *C. higginsianum* requires an interaction between a spore and the host surface, decreasing the number of available spores negatively affects fitness with respect to infection initiation. *C. higginsianum* uses RNAi machinery to control virus proliferation to prevent a deficiency in conidation, a crucial step in its lifecycle, both as a free-living organism and as a pathogen.
Arabidopsis CERK1 is not the primary chitin receptor but acts as a co-receptor with LYK5 to induce plant innate immunity

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Chitin is a fungal microbe-associated molecular pattern (MAMP) that is recognized in Arabidopsis by a lysin motif receptor kinase (LYK), CERK1. Previous research suggested that CERK1 is the major chitin receptor in plants and mediates chitin-induced signaling through homodimerization and phosphorylation. However, the reported chitin binding affinity of CERK1 is quite low, suggesting another receptor with high chitin binding affinity might be present. Here, we propose that LYK5 is the primary chitin receptor in Arabidopsis. Mutations in LYK5 resulted in a significant reduction in plant chitin response. LYK5 interacts with CERK1 in a chitin-dependent manner. Chitin binding to LYK5 is indispensable for chitin-induced CERK1 phosphorylation. The data suggest that CERK1 is not the primary plant receptor for chitin, but, instead, associates with LYK5 to mediate chitin recognition and the induction of plant innate immunity.
Characterization of root fungal endophyte community of cereal grains using 454 pyrosequencing techniques.

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Root associated fungal (RAF) endophyte symbiosis can play a vital role in development and performance of their host plant. These RAF endophytes have been found to be an important component of the root biological community especially in stressful and arid environments. We are interested to examine how different soil-type such as desert soil and clay soil influence the colonization root fungal endophytes in these grains. We also plan on comparing the fungal diversity in roots of common cereal grains wheat, maize and their respective progenitors. We employed both culture dependent and independent method of endophyte characterization. The culture independent method involved 454 pyro-sequencing of the Internal Transcriber Spacer (ITS) region of fungal ribosomal DNA. The sequences obtained were cleaned, assembled and clustered into taxonomic groups at 96.5 percent similarity using Sequencher 5.2 software and blasted in the NCBI database for characterization. The Multi dimensional comparison of Morisita horn similarities values indicates that soil plays the primary effect in root fungal colonization and a secondary effect would be the plant host, even when the plant host is a conspecific.
Dissecting the Molecular Pathway of the “RNAi-to-RdDM” Transition Responsible for Silencing Transposable Elements

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Transposable elements (TEs) are sequences of DNA that possess the ability to mobilize from one location of a host genome to another. To repress the mutagenic potential of TEs, the eukaryotic genome has evolved various defense mechanisms to silence their activity. Two distinct molecular pathways in the model organism Arabidopsis thaliana work to establish heritable epigenetic repression of TEs. The first pathway, RNA interference (RNAi), degrades the mRNA intermediates of expressed TEs into 21-22 nucleotide endogenous small interfering RNAs (siRNAs). The second pathway, RNA-directed DNA Methylation (RdDM), is targeted by 24 nucleotide siRNAs and functions to establish and maintain epigenetic silencing of TEs via cytosine DNA methylation. However, how an active TE that is targeted by RNAi transitions to an epigenetically silenced TE targeted by RdDM has remained enigmatic. We have shown that 21-22 nucleotide small interfering RNAs generated by the RNAi pathway are necessary for expression-dependent DNA methylation of TEs through a pathway we have termed RDR6-RdDM. Through the use of multiple techniques, I have identified several components of this RDR6-RdDM pathway which are required to initiate the first rounds of DNA methylation and epigenetic silencing of active TEs.
Agrobacterium-mediated transformation is negatively regulated by a Myb transcription factor via the cytokinin-signaling pathway and a membrane protein important for bacterial attachment: Application to crop transformation

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We have identified MTF, a myb transcription factor that negatively regulates transformation of Arabidopsis. MTF is down-regulated by cytokinins, resulting in increased transformation susceptibility. Nopaline-type Agrobacterium strains synthesize and secrete cytokinins via the Ti-plasmid-localized Tzs gene, whereas all strains secrete cytokinins via breakdown of isopentenyladenylated tRNAs. In plants, cytokinins trigger a signaling cascade mediated by a two-component phosphorelay pathway consisting of the AHKs (Arabidopsis histidine kinases) and the ARRs (Arabidopsis response regulators). ahk3 and ahk4 mutants show attenuated transformation, indicating involvement of these primary cytokinin receptors in transformation. Of the several ARR mutants tested, only arr3 show decreased transformation-susceptibility. One of the earliest transformation events is the attachment of bacteria to plant cells. In the hyper-transforming mtf mutant, one of the transcriptionally up-regulated genes is AT14a, which encodes an integrin domain-containing protein. AT14a is plasma membrane-localized and may mediate connections between the cell wall and the cytoskeleton. mtf mutants show increased bacterial attachment and transformation, whereas at14a mutants show lower Agrobacterium attachment and transformation. AT14a transgenic plants also show increased transformation and bacterial attachment. Thus, modulation of MTF expression via the cytokinin signaling pathway plays an important role in Agrobacterium-mediated plant transformation via increased bacterial attachment.
Transgenic maize plants expressing the Totivirus antifungal protein, KP4, are highly resistant to corn smut

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The smut fungi are important agricultural pathogens responsible for farm yield losses. Yield loss due to corn smut is generally kept below 2% with the available partially resistant varieties. However, considering that maize is the most economically important crop in the US, even a small yield loss takes on major significance. Therefore, there is an urgent need to design strategies to increase resistance of maize to smut and other fungal diseases. In the present work we expressed the antifungal protein KP4 encoded by dsRNA totivirus UMV4, in maize plants in order to increase their resistance to corn smut. The obtained transgenic maize plants expressed high levels of KP4 in their apoplasts with no apparent negative effect on plant growth and general development. Moreover, these transgenic lines displayed strong resistance to U. maydis challenges to both stem and ear tissues. These results suggest that corn smut and probably other fungal diseases can be completely controlled with the expression of this family of antifungal proteins.
Defensins are small, cysteine-rich antimicrobial proteins present in all plants. MtDef4 from *Medicago truncatula* is an evolutionarily conserved plant defensin that exhibits strong antifungal activity towards an array of fungal pathogens including the wheat pathogen *Fusarium graminearum*. In order to fully harness the potential of this protein for bioengineering crops with robust resistance to fungal and oomycete pathogens, it is essential to understand its structure-activity relationships and mode of antifungal action (MOA). MtDef4 is internalized into fungal cells through an energy-dependent process and accumulates in vesicular bodies. Structural analysis of the protein revealed the presence of a positively charged \( \gamma \)-core motif composed of \( \beta_2 \) and \( \beta_3 \) strands connected by a positively charged RGFRRR loop. Mutations of the RGFRRR sequence abolished the ability of MtDef4 to enter fungal cells, suggesting that the RGFRRR loop is a translocation signal required for the internalization of the protein. MtDef4 binds strongly to phosphatidic acid (PA) via the RGFRRR loop. Phosphatidic acid is generated from phosphatidylcholine via the phospholipase D enzyme (PLD). Three genes code for PLDs in *N. crassa* as well as in *F. graminearum*. The deletion mutant of one of these pld genes in *Neurospora* (NCU03955) is significantly resistant to MtDef4 and is unable to internalize the defensin. Analysis of the amino acid sequence of this gene reveals that it contains a Pleckstrin homology (PH) domain and a PX domain which are involved in binding to phosphatidylinositol lipids and targeting of proteins to appropriate cellular compartments. These domains are absent in the other two PLDs suggesting that this protein somehow facilitates the entry of MtDef4 into fungal cells and subsequent antifungal activity through its PX and PH domains. Microarray analysis was further used to determine the cellular targets of MtDef4. Several genes that were up-regulated upon challenge of the fungus with MtDef4 were selected for further studies to determine their contributions to MtDef4 mode of antifungal action. Deletion mutant of a gene encoding flotillin-domain containing protein was found to be resistant to MtDef4. Further characterization indicated that the entry of MtDef4 was blocked in this mutant suggesting that flotillin-like protein is involved in facilitating entry of MtDef4 into fungal cells. We have also expressed MtDef4 in transgenic wheat and found that the resulting plants were highly resistant to leaf and strip rusts.
The Arabidopsis E3 Ubiquitin ligase PUB regulates AtLYK5 protein level

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Chitin is a major component of fungal cell wall and long-chain chitooligosaccharides (degree of polymerization=6-8) are recognized as a typical fungal microbe-associated molecular pattern (MAMP) in plants. Recently, we identified the main chitin receptors in Arabidopsis. Recognition of chitin by lysin motif receptor kinase 5 (AtLYK5) induces the formation of a complex with chitin elicitor receptor kinase 1 (AtCERK1), leading to the activation of AtCERK1 intracellular kinase domain, then induces plant innate immunity. However, how the protein turnover of AtLYK5 and AtCERK1 is regulated by chitin is still unclear. In this study, we found chitin induced AtLYK5 protein accumulation, but triggered AtCERK1 protein degradation. Chitin-triggered AtCERK1 degradation is mediated by 26S proteasome. Interestingly, basal AtLYK5 protein level is also controlled by 26S proteasome. Our results suggested there were two different E3 ubiquitin ligases regulating LYK5 and CERK1 degradation, respectively. One plant U-Box E3 ubiquitin ligase (PUB) was identified for regulating basal LYK5 protein level. Knockout mutants of this PUB showed hypersensitivity in chitin-induced early innate immunity, such as the production of reactive oxygen species (ROS) and MAP kinase phosphorylation, but exhibited normal responses in chitin-induced late innate immunity, such as gene expression and callose deposition.
Screening switchgrass endophytic isolates for bacteria capable of associative nitrogen fixation

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Switchgrass (Panicum virgatum L.) is a perennial C4 grass native to North America that is being developed as a feedstock for cellulosic ethanol production. Industrial nitrogen fertilizers enhance switchgrass biomass production but add to production and environmental costs. Biological nitrogen fixation is a potential sustainable alternative source of nitrogen. We have a collection of almost 550 pure endophytic bacterial cultures isolated from the roots of switchgrass plants native to various parts of northern Oklahoma. In this project, we are screening our entire collection for bacteria capable of associative nitrogen fixation. The presence of the nitrogenase reductase gene (nifH) is confirmed in 60 isolates by a PCR-based method. At present, these isolates are being tested for functional nitrogen fixation in association with switchgrass in a greenhouse experiment. We have two promising isolates showing signs of associative nitrogen fixation. Bacteria possessing the capability of nitrogen fixation under these conditions will be used for future growth promotion studies.
Do Plants Use Mechanical Signals To Sense Pathogens?

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A great deal is known about the signaling pathways that plants employ to sense and respond to molecular patterns associated with pathogenic infection. For example, plant cells specifically recognize molecules that result from cell wall damage (triggering DAMP-triggered immunity), pathogen-associated molecules (triggering PAMP-triggered immunity), and pathogen-derived effector proteins (triggering effector-triggered immunity). We are interested in a related but less well-studied question: Do mechanical signals also contribute to plant-pathogen interactions? In addition to the established signals listed above, mechanical signals such as increased membrane tension (e.g. caused by the formation of a fungal appressorium) could also be used as a signal by the plant. Increased membrane tension can be detected by mechanosensitive ion channels. Here we present several lines of evidence supporting the hypothesis that the Arabidopsis mechanosensitive ion channel MscS-Like (MSL)10 is involved in immune response signaling: 1) Overexpression of MSL10 is associated with reduced cell expansion, H₂O₂ accumulation, and cell death; 2) these phenotypes are relieved by growth at high temperatures; and 3) these phenotypes are associated with immune response-related gene expression patterns. We believe this work is a first step toward understanding how mechanical signaling may be used by plants to sense pathogen infection.
Comparative analysis of virus-derived small RNA profiles of cassava brown streak viruses in infected cassava plants

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Infection of plant cells by viral pathogens triggers RNA silencing, an innate antiviral defense mechanism mediated by Dicer-like proteins. Small RNAs are produced that associate with Argonaute-containing silencing complexes which act to inactivate viral genomes. Deep sequencing was used to compare virus-derived small RNAs (vsRNAs) in cassava genotypes NASE3, TME204 and 60444 infected with the +ssRNA viruses, Cassava brown streak virus (CBSV) and Ugandan cassava brown streak virus (UCBSV). An abundance of 21-24 nt vsRNA was detected covering the entire CBSV and UCBSV genomes. 21-nt vsRNA were predominant followed by the 22-nt class with a slight bias toward sense compared to antisense polarity. Distribution and frequency of vsRNAs differed between the cassava genotypes and viral genomes. Cultivar 60444 accumulated the highest levels of vsRNAs followed by TME204. vsRNA against UCBSV were seen in greater abundance than CBSV in infected cv.60444 with the opposite observed in TME204. NASE3 was resistant to UCBSV infection, and accumulated very low CBSV-vsRNAs. Irrespective of genotype or viral infection, cytoplasmic inclusion and P3 proteins genes produced maximum vsRNAs in infected cassava, respectively. Our results indicate disparity between CBSV and UCBSV host-virus interaction mechanisms, and provide insight to occurrence of CBSD resistance.
Effects of antibiotics on the murine vagina microbiome with GBS colonization and the role of glutathione

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Group B Streptococcus (GBS) is the leading cause of neonatal meningitis. GBS colonizes the female vagina where it can be transmitted to the infant. Pregnant women who test positive for GBS are given antibiotics during pregnancy to eliminate GBS, also killing beneficial bacteria, which could have effects on the immunity of the infant. If GBS can be eliminated without having to clear beneficial bacteria, then the infant will have a lower risk of both acquiring GBS and developing immune disorders later in life. Glutathione could be beneficial to colonization because of its antioxidant properties. The focus of the current study is the effects of antibiotics on the vagina microbiome with and without GBS colonization and the role of glutathione in GBS colonization. Mice (treated with antibiotics or not) will be colonized in the vagina with GBS at varying ages, and will be tested for the presence of GBS by sequencing 16s rDNA. In order to determine glutathione’s role in colonization, the experiment will be repeated comparing colonization with either glutathione-deficient or wild type GBS. Results will show how the vaginal microbiome is changing in response to antibiotic treatment and how glutathione helps GBS colonize the mammalian vagina.
Evaluation of virus induced gene silencing to identify and characterize host genes involved in endophyte-grass interactions

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Epichloë species (Clavicipitaceae, Ascomycota) are symbiotic fungi well known for their association with many cool-season grasses. Endophyte-host interactions contribute to plant growth promotion, protection from many pathogens and insect pests, and tolerance to drought stress. Although these important endophyte-grass systems have been extensively studied, little is known about the molecular mechanisms involved in the interactions between host and endophyte. In particular, the host targets and/or signaling pathways from the plant are not elucidated despite the potential to understand symbiotic biology and apply this knowledge for forage improvement. Here we evaluated an approach to identify and characterize host genes associated with symbiotic interactions using virus-induced gene silencing (VIGS). Genes encoding phytoene desaturase (PDS) and green fluorescence protein (GFP) were tested to establish and evaluate VIGS methodology using Lolium arundinaceum (tall fescue). Delivery of the Brome mosaic virus silencing vector to leaves of several tall fescue lines via sap extract after virus amplification in maize was effective. We are exploring the impact that endophyte presence has on virus infection efficiency. Once conditions are fully optimized for effective VIGS in the presence of the endophyte, selected candidate host genes will be characterized to explore the molecular mechanisms of endophyte-grass interactions.
Expression of potential pathogenicity determinants during compatible and incompatible interactions between *Colletotrichum graminicola* and maize

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*Colletotrichum graminicola*, is the causal agent of anthracnose leaf blight (ALB) and anthracnose stalk rot (ASR). ASR is the most devastating phase of the disease, and the industry estimates it is responsible for annual yield losses of 5-10% in corn. *C. graminicola* is a hemibiotroph; it initially invades its host while it is alive, and then switches to destructive necrotrophic growth and the host is killed. Previously, we identified a non-pathogenic *C. graminicola* mutant (MT), with an insertion in a gene predicted to encode one of the components of the signal peptidase complex. This mutant, is similar to the wild type (WT) *in vitro* and during early host penetration, but fails to switch to necrotrophy. We determined that when the MT was co-inoculated with the WT in maize leaf sheaths, the MT strain was able to colonize the host. Our results suggest that *C. graminicola* produces diffusible compounds during colonization that predispose neighboring cells nearby living host cells for fungal invasion, and that the MT is non-pathogenic because it is impaired in the production of one or more of these substances.

To identify potential candidates for these compounds that are critical for establishment of compatibility, we used whole transcriptome analysis of maize leaf sheaths inoculated with WT and MT strains. Analysis of WT-inoculated sheaths indicated that a total of 760 fungal genes were differentially expressed during the transition from pre-penetrating appressoria, to biotrophic stage and 992 during the switch to necrotrophy. In comparison, only 20 genes were found to be differentially expressed in the transition to biotrophy in the MT. Our results suggest that the MT strain stops growing soon after penetration and fails to establish a successful biotrophic interaction. Comparisons between genes induced in WT and MT, provided various candidates, including some encoding potential secreted effectors, that may function in the establishment of *C. graminicola* biotrophy and switch to necrotrophy. Further characterization of these genes, their products and their host targets could lead to the development of novel therapies to manage this disease.
Influence of plant host species on the root associated bacterial community and functional redundancy of the root microbiome under phosphate starvation.

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Plants harbor a specific and complex microbiota at the surface of the root (rhizosphere) and within the root (endophytic compartment) which influences plant health and productivity. Various factors such as soil type, abiotic/biotic stress, host developmental stage, and host species will shape the root microbiome. The extent to which the host selects its root microbial community remains poorly understood. To determine the influence of plant host species on the assembling of both the rhizospheric and endophytic microbiome, we are comparing the well-studied bacterial root bacterial microbiome of Arabidopsis thaliana Col-0, with those of two monocot species Brachypodium distachyon Bd21 and Setaria viridis A10-1 from plants grown on the same wild soil using high-throughput 16S ribosomal RNA profiling. The Dangl laboratory has isolated ~600 bacterial strains from the rhizoplane and endophytic compartments of Arabidopsis thaliana and is evaluating the effect of these isolates on plant health under various nutrient starvation stresses. This bacterial collection is currently being tested on Setaria viridis focusing on the identification of isolates that rescue phosphate starvation stress to establish to whether the core microbiome may also present functional conservation across species. We are in parallel establishing a collection of bacterial strains isolated from the root of Setaria viridis to refine our characterization of both the core and potential species-specific microbiome.
Proteomics & Mass Spectrometry: Essential tools for systems biology

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Nowadays mass spectrometry (MS) is well established as an integrated tool for studying biological systems, in particular in the identification and quantification of perturbations from the environment, such as host-microbe interaction and the adaptive and/or defensive mechanisms induced. Protein and metabolite contents which are the final products of genome expression correlate intrinsically with how different species and strain genotypes interact with the environment. Qualitative MS-based proteomics tools are routinely used in defining protein-protein interaction networks and protein signaling pathways, whilst quantitative methods can reveal the dynamics of cellular networks in response to modifications with the environment. Though proteomic approaches are more advanced, approaches are rapidly developing for the study of metabolites and metabolic networks with the emergence of higher resolution mass spectrometers. The Proteomics & Mass Spectrometry Facility (PMSF) has been involved in facilitating the progress of various research projects for profiling proteins and targeted small molecules from different species and strains under various conditions. The main tools used for protein and small molecule analyses and the state of the art equipment at the PMSF are presented here.
Comprehensive phosphoproteome characterization in *Chlamydomonas reinhardtii* using complementary HPLC fractionation methods followed by shotgun LC-MS/MS.

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Differing technologies for the characterization of phosphopeptides by mass spectrometry often purport to offer the same broad outcome. When particular phosphorylation sites are not found in a sample, it is often the sensitivity of the mass spectrometer that is first called into question. Here we show that steps upstream of the mass spectrometer can be equally as important in determining if phosphorylation sites of interest appear in a final dataset. The approach uses a single protein extract from *C. reinhardtii* cells from which a single phosphopeptide pool has been derived and aliquoted. Two commonly employed sub-fractionation methods were then compared prior to peptide identification by Orbitrap mass spectrometry: hydrophilic interaction chromatography (HILIC) and strong cation exchange (SCX) chromatography. For a dataset of >16,000 phosphorylation sites, our results conclude that the two methods are highly exclusive for certain types of peptide with overlap as little as 40%. Since different enrichment approaches also bias phosphopeptide pools in terms of phosphorylation sites found per peptide, clearly phosphopeptide experiments need careful design from the outset.
Using RNAseq to Discover Novel Regulators and Genes in Nitrogen Utilization Pathways.

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Nitrogen is a key limiting plant nutrient and its availability is expected to have significant impacts on the expression of genes that function in nitrogen metabolism and growth responses to N. Although a key target for improving maize yield response to nitrogen, relatively little is known about the gene regulatory systems that modulate N remobilization. RNA expression profiling experiments were conducted to identify nitrogen-responsive genes in the maize B73 genome. A curated set of genes known to function in nitrogen metabolism were used to validate expected expression patterns and identify the specific nitrogen-responses within these pathways. These key N metabolism enzymes were also the basis for discovering candidate regulators of nitrogen metabolism by finding genes which are co-expressed with these enzymes. The Pearson’s R coefficient was calculated for all expressed genes to identify collinear expression patterns between genes. Interestingly, some of the best correlated genes are found within 10kb along the same strand of DNA, and may be alternative splice variants of a single gene. With a threshold of R>0.9, 111 putative alternative splice variants were identified, and twelve were experimentally tested using PCR of leaf cDNA. Ten of these were shown to be co-transcribed.
Biological Nitrogen Fixation in Legumes

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Nitrogen is one of the growth limiting nutrients for plants. Many plants receive the nitrogen artificially through fertilizer which requires extensive energy such as fossil fuels to make and after use can cause pollutants in the form of runoff material. Legumes, i.e. soybeans, Lotus japonicus and Medicago truncatula produce their own nitrogen by forming a special root derived organ called nodule by the infection of host specific symbionts. An analysis of M. truncatula mutant having a Tnt1 insertion shows a strong nodulation phenotype, as well as growth defects such as shorter roots and shoots. In a rare event, the mutant can form a very few, small nodules. Thus, the mutant is unable to fix nitrogen. In contrast, the wild type plants are able to form successful N₂-fixing nodules and hence plants become larger, healthier, and have thicker roots. Identification and functional characterization of the causal gene will uncover the mechanism of nitrogen fixation ability.
Host species-dependent and seasonally variable microbial communities in the carnivorous pitcher fluids of *Sarracenia purpurea* and *S. psittacina*

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Carnivorous pitcher plants have modified tubular leaves that accumulate captured insects, plant secretions (e.g., digestive enzymes) and water from rain or flooding of the habitat. Pitcher fluids can serve as a model to study food web dynamics, community genetics, trophic interactions, succession, and population structure. We investigated differences in the microbial community richness and composition in the pitcher fluids of two *Sarracenia* species using 454 sequencing of rRNA gene amplicons. Pitcher plants were sampled during spring, summer and fall at Splinter Hill Bog in Alabama and Ponce De Leon Bog in Florida. Eubacterial phylotypes from pitchers sampled during spring and summer showed dramatic season-dependent differences, but no location-specific differences. The summer samples from *S. psittacina* and *S. purpurea* pitchers formed separate clusters in principal co-ordinates analysis, indicating a strong effect of the plant species. Much greater abundances of *Rhodopseudomonas* and *Bacillus* were found in *S. psittacina* compared to *S. purpurea* pitchers, while *Pseudomonads* were much more abundant in *S. purpurea* pitchers. The dominant archaeabacterial phylotypes were related to halobacteria and methanobacteria, but most belong to a previously undiscovered archaeabacterial class. Among eukaryotes, apicomplexan alveolates were more common in *S. purpurea* than in *S. psittacina*. Ants (Family:Formicidae), the major food source for these carnivorous plants, were found to represent primarily one phylotype in *S. psittacina*, but numerous phylotypes in *S. purpurea*, indicating that these two plant species have adopted respective specialist and generalist predatory niches. Numerous and dynamic rotifer, mite and fungal phylotypes were also detected. Keywords: archaeabacteria, carnivorous plants, community structure, metagenomic analysis, microbiome
Cyanobacterial Alkanes Enable Low-Temperature Growth

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Aliphatic hydrocarbons (C₁₅-C₁₉) are universal cyanobacterial metabolites. N-heptadecane is most common, but methylated or unsaturated hydrocarbons also exist. These metabolites were identified nearly 50 years ago¹, but their universality² and biosynthetic pathways³-⁴ are recent discoveries. We have added their functional significance to this list of new insights. Heptadecane enables Synechocystis 6803 to grow at low temperature, likely by maintaining membrane fluidity. We are using this and other functional insights to guide engineered photosynthetic overproduction of this alternative diesel fuel.
Heterotrimeric G-protein mediated signaling regulate soybean nodulation

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Symbiotic nitrogen fixation provides a sustainable channel for the release of nitrogen into the biosphere and accomplishes the requirement for agricultural nitrogen fertilizer. Legume species form a symbiotic relationship with rhizobia and this relationship is fashioned following the exchange of a series of signals, eventually resulting in the formation of specialized root organ, nodule. The availability of sequenced genome and a variety of functional genomics approaches have resulted in identification of multiple signaling pathways that are involved during nodule development in soybean.

Heterotrimeric G protein mediated signaling is an important aspect of transmembrane signal transduction in all eukaryotic organisms. Plants, despite having fewer G-protein components use this signaling pathway to regulate multiple growth and development processes. We have identified an elaborate G protein family in soybean, consisting of 4 Ga, 4Gb, 10 Gγ and 2 RGS proteins. All G protein genes are expressed in nodules and hairy roots of soybean. We have recently shown that specific G protein subunits exhibit significant changes in expression at different time points after Bradyrhizobium infection during nodule formation. Moreover, the expression of some G protein genes was considerably changed in supernodulating and nonnodulating soybean mutants indicating expression of those genes are biologically relevant during nodulation. RNAi-mediated suppression of specific G protein components results in significant reduction of nodule number and change in nodule morphology. Expectedly, overexpression of G protein genes also leads to altered nodule numbers and phenotype in transgenic hairy root of composite plants. These results reveal that G protein-mediated signaling pathways play important role during soybean nodulation. Our current works focuses on the mechanism of G-protein signaling and its regulation during nodule formation.
Setaria viridis as a Model System to Study Jasmonate Signaling in Bioenergy Grasses

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Plants routinely encounter stresses that often lead to compromised biomass and yield. In order to successfully produce offspring while responding to external stresses, it is crucial for plants to have a fine-tuned response network to control the balance between growth and defense. Jasmonate (JA) is an important phytohormone that regulates both stress responses and growth and development. JA signaling outputs are tightly regulated by JASMONATE ZIM-DOMAIN (JAZ) proteins through interacting with diverse transcription factors. Hence understanding the JAZ signaling network will provide key information on how JA can control multiple signaling outputs to promote defense responses and inhibit growth. Here, we use the emerging panicoid model Setaria viridis to study JA signaling. Setaria viridis is closely related to bioenergy crops including switchgrass, Miscanthus, sorghum and maize. We show that Setaria growth is inhibited upon exogenous application of JA, and that JAZ genes are rapidly induced upon wounding across Setaria leaf segments. Screening of chemically-induced NMU mutant lines also led to identification of three mutant lines that are hypersensitive to exogenous JA treatment. Outcomes from this research provide insight to how bioenergy crops can be engineered to defend against stresses without compromising biomass production.
Analysis of a Cyst Nematode Effector Protein Targeting Host Nuclear Functions

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Cyst nematodes are a group of plant-parasitic nematodes of great economic importance. These obligate biotrophs establish an enlarged, multinucleate and metabolically hyperactive feeding site called a syncytium from which they derive nutrients necessary to complete their life cycle. The nematode uses its hollow mouth spear or stylet to deliver effector proteins into host cells. These stylet-secreted effectors (SSEs) manipulate host cellular processes and promote parasitism. Several SSEs have been identified from the soybean cyst nematode Heterodera glycines and the corresponding orthologs were cloned from the beet cyst nematode H. schachtii to facilitate functional studies in Arabidopsis. One such SSE protein called 30D08, is a novel protein with a predicted nuclear localization signal in the mature protein. Transient expression assays in onion and tobacco epidermal cells confirmed 30D08 localization to plant cell nuclei. Silencing of 30D08 in nematodes by plant-mediated RNAi reduced infection. A yeast two-hybrid screen identified a specific interaction of 30D08 with a nuclear-targeted host protein involved in pre-mRNA splicing. The target protein was confirmed to be expressed in feeding sites and knock-out mutants showed increased resistance to nematodes. Expression of 30D08 under the control of the host target promoter resulted in shoot and root growth promotion in young seedlings. RNAseq analysis of 30D08 overexpression lines revealed differential regulation of a subset of genes controlling several key cellular processes important for syncytium formation. Our studies suggest that 30D08 is targeted to the plant nucleus where its interaction with a host protein involved in pre-mRNA splicing may regulate the expression of genes important for feeding site formation.
Host plant resistance to cassava-infecting geminiviruses: What role for RNA silencing in antiviral defense?

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Cassava mosaic disease (CMD) complex continuously impacts cassava production in tropical Africa. Breeding for resistance against the causal geminiviruses has focused on introgression of the polygenic locus (CMD1) and the dominant monogenic locus (CMD2). However, the mechanism(s) of resistance imparted by CMD1 and CMD2 remain unknown. Susceptible and resistance cassava cultivars were challenged by microparticle bombardment with infections clones of African cassava mosaic virus (ACMV-CM) and East African cassava mosaic virus (EACMV (K201)). Cassava genotypes carrying CMD1 or CMD2 locus did not develop systemic infection when inoculated with ACMV, and virus titer was undetectable by PCR beyond 7 days post inoculation (DPI). In contrast, genotypes developed severe systemic disease symptoms and accumulated high virus titer when challenged with EACMV (K201), with resistant genotypes reverting to become asymptomatic by 65 DPI with undetectable virus load in new leaves. Northern blotting confirmed presence of virus-derived siRNAs (vsRNA) and their correlation with virus titre, symptom severity and recovery from disease symptoms in resistant cultivars. Deep sequencing confirmed production of 21-24-nt vsRNA covering the entire viral genome sequences in both polarities. Experiments are ongoing to determine what role the RNA silencing pathway plays within the CMD1 and CMD2 resistance mechanisms.
Targeting isoprenoid metabolism in the plastid derived MEP pathway in the malarial pathogen *Plasmodium falciparum*.

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Isoprenoids are the most diverse class of metabolites found in nature and include such vital compounds as chlorophyll, ubiquinone and cholesterol. The synthesis of these compounds are essential and occur through one of two independently evolved pathways, the mevalonate pathway in humans and all metazoans, and the methylerthritol phosphate (MEP) pathway in plant chloroplasts, Gram negative bacteria, and the malaria pathogens in the *Plasmodium* spp. The presence of these two distinctive pathways makes the MEP pathway a tempting target for both antimicrobial and herbicidal development. To explore this potential, a collaborative effort between Washington University and the University of Liverpool used a chemoinformatic strategy to identify 4,750 compounds, which were tested for the capability to inhibit the third enzyme in the MEP pathway (IspD). This collaboration has developed a class of compounds that inhibit the enzyme and the parasite at concentrations of less than 500 nM. Mutational and biochemical analysis demonstrate the mechanism of enzymatic inhibition. These results support the potential for the MEP as a productive target for both anti-microbial and herbicidal development. In addition, our novel compounds have the potential as lead antimalarial compounds that could become a part of future treatment regimes.