



2013 APS-MSA Joint Meeting Abstracts of Presentations

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Interception and identification by deep sequencing of a “caulimo-like” virus in a potato germplasm accession imported from South America

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A small portion (3%) of seedlings germinated from botanical potato seed accession JCM-23 imported from South America showed severe upper leaves deformation and necrosis. The diseased plants tested negative for most of the known viruses by bioassay, electron microscopy (EM), ELISA and nucleic acid-based technology. Presence of isometric particles by EM analysis suggested an unknown virus as pathogen. The virus induced a mild but conspicuous systemic vein clearing only on *Nicotiana tabacum* cv. Samsun plants, indicating that it is difficult to transmit mechanically. However, the virus was readily transmitted to potato and tomato plants by grafting. Tubers harvested from infected plants did not show any symptoms, but plants grown from these tubers developed necrosis, leaf deformation and rugosity (‘frog’ skin) as secondary symptoms. The diseased plants were subjected to small-RNA deep-sequencing analysis. BLAST search against virtual viral sequences showed that several contigs larger than 700 bp had identities higher than 90% with *Cauliflower mosaic virus*, suggesting the presence of a “caulimo-like” virus. The viral fragment was further amplified from the diseased plants by conventional PCR with specific primers designed from the contigs. This virus is a potentially dangerous seed-transmissible pathogen infecting potatoes and the USDA-APHIS-PPQ Plant Germplasm Quarantine Program prevented the introduction of another putative unknown foreign potato pathogen into the USA.

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Field evaluation of promising breeding lines and varieties of common bean for tolerance to soilborne pathogens

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This study was conducted to identify and incorporate sources of resistance to major root pathogens into adapted bean varieties being developed in a collaborative Dry Grains PULSE CRSP project, with a focus on Ecuador and Rwanda. The use of varieties resistant to root pathogens is the most effective and practical strategy for managing root diseases of common bean. Field trials of promising lines from the Michigan State U. and ARS-Puerto Rico breeding programs were evaluated in the bean root nursery at the Vegetable Research Farm, NYSAES near Geneva, NY over the duration of the project. This site is heavily infested with *Fusarium solani* f. sp. *phaseoli*, *Pythium ultimum*, *Rhizoctonia solani*, and *Thielaviopsis basicola*. In 2011, 33 lines and varieties were arranged in a randomized block design with 4 replications. Each plot consisted of two rows, 7 m long and 0.75 m apart. All maintenance practices were according to recommended commercial guidelines. Root rot severity, among the lines tested varied greatly, ranging from 3.4 (10IS-6567, Eldorado pinto, Zorro black) to 6.0 (CLRK) on the 1 (healthy) to 9 (late stages of decay) scale. Also, many of the tested lines exhibited excellent vigor, productivity and high tolerance to a severe incidence of common bacterial blight, including Eldorado, RR008, RR016, RR005, 10IS-6480. Similarly, 27 bean lines and varieties were evaluated in 2012 and root rot severity ratings obtained ranged from 3.2 (Medalist) to 5.8 (Pink Panther).

Analysis of 3'-terminal region of Papaya ringspot virus-W isolates from southern United States

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Papaya ringspot virus-W (PRSV-W) is one of the most common viruses infecting cucurbits in southern United States. Fifty eight PRSV-W isolates were collected from four different States including Arkansas, Florida, Oklahoma and Texas. 3'-terminal region (including N1b and CP genes) were amplified and sequenced in these fifty eight isolates. This study showed that PRSV-W isolates in southern United States shared identity ranged from 92.2-99.9% and 94.9-99.6% at nucleotide and amino acid levels respectively, in the

Mortierella elongata belongs to a group of basal fungi (*Mortierellomycotina*) and is commonly isolated from forest soils and healthy plant roots. Recent reports indicate that some isolates of *M. elongata* host endosymbiotic bacteria, but it is unclear whether these are lineage-specific associations. Given the geographically widespread distribution of *M. elongata* and its ubiquitous presence in forest soils and plants we chose to sequence its genome through the JGI Forest Metatranscriptome CSP. We also sought to assemble the genome of the bacterial endosymbiont. The 50 Mb genome of *M. elongata* was sequenced to 112x coverage. Of the 220,113 putative proteins identified in *M. elongata*, only ~50% have orthologs in other fungal species having sequenced genomes). The *M. elongata* genome appears to be enriched in genes related to lipid metabolism (e.g. sphingolipids, etherlipids, and glycerophospholipids), tryptophan metabolism, siderophore group nonribosomal peptides, and glucan 1,4-alpha glucosidases compared to genome sequences of other basal fungi. The endosymbiotic bacterium sequenced along with the *M. elongata* isolate is related to *Glomeribacter* (endosymbiont of *Gigospora*, *Scutellospora*) within the Burkholderiaceae. The ~2.6 MB endosymbiont genome is larger than that of *Glomeribacter* but reduced compared to free-living *Burkholderia*. Although many genes have been lost, some gene families have expanded including those involved in protein metabolism and electron transport.

Characterization of *Arabidopsis* CRT1 in plant immunity and genome stability

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A genetic screen for components involved in resistance (R) protein-mediated immunity in *Arabidopsis* led to isolation of *crt1* (compromised recognition of TCV). CRT1 was shown to be a MORC ATPase/endonuclease that physically interacts with multiple immune components. While CRT1 is mainly located in endosome-like vesicles in the cytoplasm, a subpopulation resides in the nucleus, which increases after infection. The combined findings that CRT1 i) is an endonuclease, ii) physically interacts with several components of the DNA repair and recombination (R/R) pathway, iii) is localized to heterochromatin, and iv) is implicated in epigenetic regulation, including suppression of heterochromatic transposable elements (TEs), suggest that CRT1 has an important nuclear function(s). Thus, we are investigating CRT1's role in the nucleus, particularly its involvement in stress-triggered genome stability, and to assess the importance of this function in plant immunity and evolution. To assess whether stress-triggered genome stability is regulated by CRT1, Southern blot analysis and chromatin accessibility PCR are currently being performed on consecutive generations of pathogen-inoculated wild type (WT) and mutant plants lacking CRT1 and its closest homolog CRH1.

Oxidized lipids control disease development during *Aspergillus* infection of maize

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Despite advancements in mechanical and chemical technologies, ear-rotting fungi continue to threaten the maize industry from seed maceration and mycotoxin production creating economic losses and hazards for human and animal health. Oxidized lipids have gained recent attention for their roles in regulating both plant and fungal processes. These metabolites, termed oxylipins, are potent eukaryotic endogenous signals produced through dioxygenase activity especially from the lipoxygenase (LOX) and Psi producing oxygenases (Ppo) gene families in plants and fungi, respectively. Remarkably, oxylipins from both kingdoms are biochemically similar, prompting an exciting hypothesis; during plant-fungal pathogen interactions, oxylipin signals are reciprocally exchanged between host and parasite. In this study, kernel bioassays of *lox3* and *lox5*, were performed with a diverse collection of *Aspergillus flavus* oxylipin-deficient mutant strains. Three days post infection, sporulation and colonization were determined. Results indicate these LOX isoforms have specific effects determining infection outcomes. Within this pathosystem, the unique bouquet of oxylipins generated by both host and pathogen determines the outcomes of infection. This knowledge will spearhead understanding molecular mechanism behind oxylipin-mediated signal exchange during plant-fungal interactions and may allow development of novel environmentally friendly disease resistance and prevention.

Phylogeny of mitosporic Capnodiales and description of a new sooty mold species *Fumiglobus pierisiculus* from British Columbia, Canada

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Capnodiales are sooty molds, saprotrophic fungi that grow superficially on plants, usually in association with sap-sucking insects. Our goals were to identify a new sooty mold from the ornamental shrub Japanese andromeda, and to use molecular phylogenetics to analyze patterns of character evolution of the fungus and its relatives. Morphological analysis of the pycnidial state suggested the fungus was in the genus *Fumiglobus* but it did not fit in any previously described species. We illustrate and describe the mold as *Fumiglobus pierisiculus*. We also for the first time locate the phylogenetic position of *Fumiglobus* using LSU and SSU r-DNA genes. Our analysis shows that *Fumiglobus* is an early-diverging genus within Capnodiales with strong bootstrap support. We also provide new sequence data of the type species of the mitosporic genus *Conidiocarpus*, also in Capnodiales. We confirm *Conidiocarpus* as the anamorph of *Phragmocarpus*. By rules of nomenclatorial priority, the name of the holomorph genus is *Conidiocarpus*. We comment on morphological characters that help define the Capnodiales including the pycnidial state and mucilaginous hyphae, and analyze the distribution of these characters in a phylogeny. Our analyses help provide a comprehensive molecular and morphological definition of the Capnodiales.

Identifying novel bacterial disease resistance sources for rice

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Major constraints to rice yield worldwide are bacterial diseases caused by pathogens such as *Xanthomonas oryzae* pv *oryzae* (*Xoo*) and *Xanthomonas oryzae* pv *oryzicola* (*Xoc*), causal agents of bacterial blight (BB) and bacterial leaf streak (BLS) of rice, respectively. BLS is an emergent disease, causing considerable losses in Africa and China with no known source of single gene resistance. In Africa, no effective BB resistance is available in currently used germplasm. A broad-spectrum source of resistance effective against multiple bacterial pathogens would be a powerful resource for rice breeders. We are using a second generation-mapping resource, Multi-Parent Advanced Generation Inter-Cross (MAGIC) population, to identify new sources of resistance for BB and BLS of rice. Two MAGIC populations, one from indica and one from japonica founders, were developed using eight elite cultivars with highly diverse backgrounds. The founders of each population exhibited highly differential responses to African strains of *Xoo* and *Xoc*. Screening of the populations and genome-wide association mapping using SNP markers are in progress to identify disease resistance QTL and to provide markers for rice breeders. Because the MAGIC founders are elite cultivars, ultimate use of resistance sources by breeders will be expedited, thus improving the yield of rice crops.

Yield losses in oats due to crown rust in Alabama

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Crown rust of oats, caused by *Puccinia coronata* f.sp. *avenae*, is primarily controlled with race-specific resistance. Unfortunately, increased pathogen virulence in recent years has led to yield losses in oats cultivars that had previously been designated as crown rust resistant. In 2008, 2009, and 2012, oats cultivar Coker 227 was planted in Baldwin Co., AL (southern coastal site) and treated with fungicide programs to achieve varying crown rust levels. Fungicides included pyraclostrobin (Headline 2.09EC at 6 fl. oz.), azoxystrobin (Quadris 2.08SC at 4 fl. oz.), propiconazole (Tilt 3.6EC at 4 fl. oz.), and propiconazole + trifloxystrobin (Stratego 2.08EC at 7 fl. oz.). Fungicide application timings were Feekes growth stage 9, 10.5 and dual application at both growth stages. Flag leaf rust intensity ratings on non-treated plots averaged 4.4, 3.9 and 9.7 in 2008, 2009 and 2012, respectively. Regression of rust intensity ratings done in late April on yield in each year yielded significant relationships ($P < 0.0001$) with estimated losses of 8.8 (11.9%), 9.9 (11.2%) and 4.8 (7.6%) bu/A per increment of crown rust intensity in 2008, 2009, and 2012, respectively.