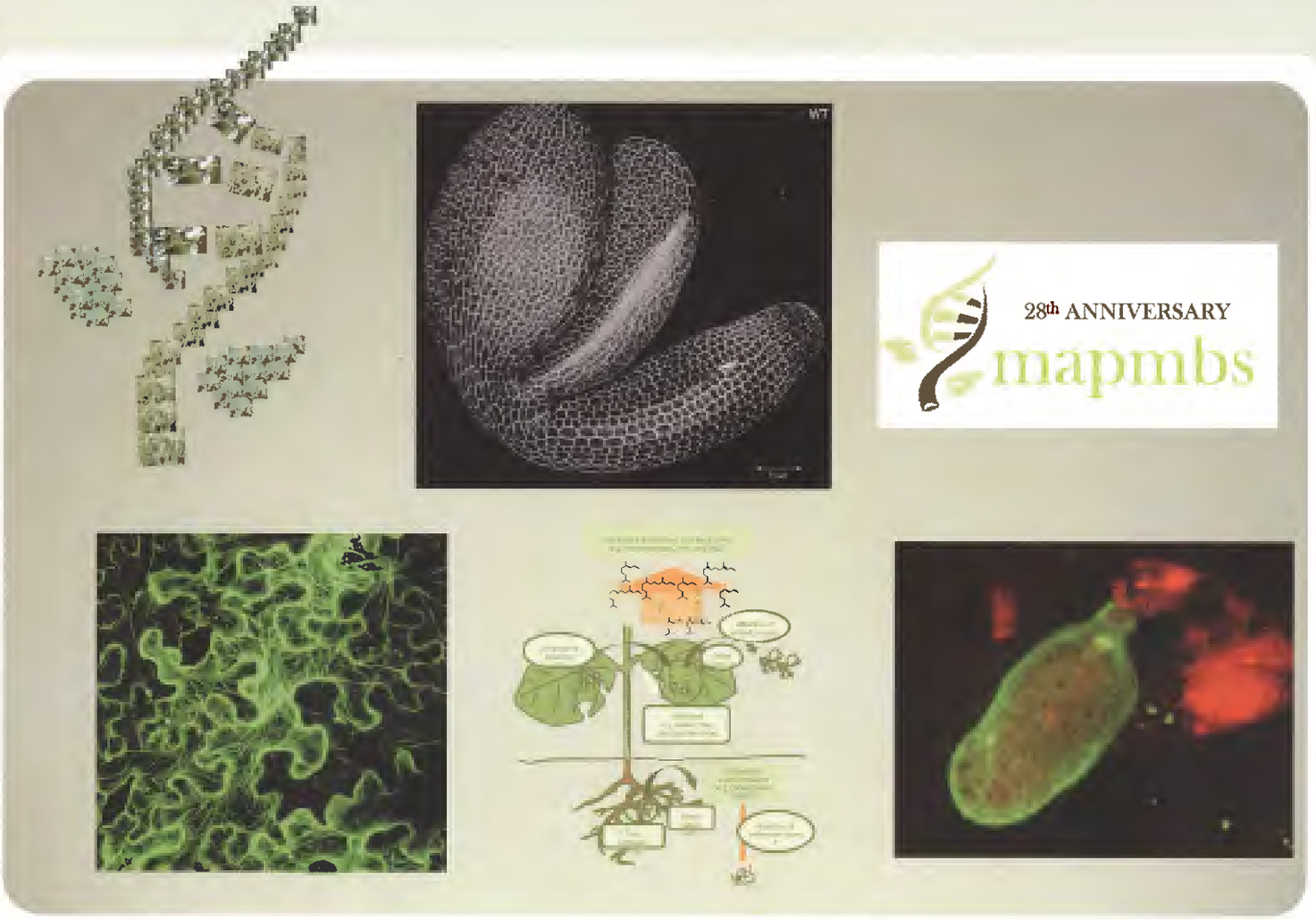

28th Annual Mid-Atlantic Plant Molecular Biology Society Meeting

August 15th & 16th, 2011

National Wildlife Visitor Center,
Patuxent Research Refuge
Laurel, MD

WELCOME

On behalf of the Organizing Committees we welcome you to the twenty-eighth annual meeting of the Mid-Atlantic Plant Molecular Biology Society in 2011. MAPMBS was formed more than 25 years ago to provide a high quality, accessible and affordable plant molecular biology meeting each year for scientists in the Mid-Atlantic region. Our goal continues to be to bring some of the best minds in the country to the Mid-Atlantic region to present interesting advances in plant molecular biology at a reasonable price and an accessible location. We hope to encourage students, post-docs, and senior scientists to actively participate in presentations and discussions. In addition, the meeting is designed to promote interaction among scientists in an informal atmosphere. Therefore, we provide on-site lunches and breaks so that each participant has the opportunity to meet invited speakers and other presenters.

The meeting encompasses a broad range of research areas, which we hope will stimulate participants by informing them of advances outside of their own immediate interests.

Please contact members of the organizing committee if you have any thoughts or comments for consideration in the planning of future meetings. Also, if you are interested, please join next year's organizing team and volunteer your services to improve upon this year's meeting. All are welcome at any stage of the planning and organizing process!

Many people were involved in the planning and organization of this meeting, and we give them our hearty thanks. We wish to especially thank our sponsors and exhibitors who furnish us with up-to-date products, and help to defray costs.

We thank you for your continued support of and participation in the Mid-Atlantic Plant Molecular Biology Society. You can keep up with MAPMBS on our website:
http://bioinformatics.towson.edu/mapmbs/default.aspx

Benjamin F. Matthews and Leslie A. Wanner

Co-chairs

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Monday August 15, 2011

9:00 **registration and poster set-up**

9:20 **Welcome** Ben Matthews and Leslie Wanner

Session I **GENE DISCOVERY, LARGE-SCALE and SMALL**
Moderator, **Bret Cooper,** USDA-ARS Soybean Genomics & Improvement Lab, Beltsville, MD

9:30 **Kerry Pedley**, USDA- ARS Foreign Disease-Weed Science Research Unit, Fort Detrick, MD
Identification of soybean genes involved in resistance to Asian soybean rust using virus-induced gene silencing

10:05 **Arianne Tremblay**, USDA-ARS Soybean Genomics & Improvement Laboratory, Beltsville, MD
Discovery of new soybean and soybean rust genes using next generation sequencing

10:30 **Coffee break: Poster session and Exhibitors**

Session II **GLOBAL GENE REGULATION IN PLANT DISEASE**
moderator, **David Puthoff**, Department of Biology, Frostburg State University, Frostburg, MD

11:00 **Shunyuan Xiao** Institute for Biosciences & Biotechnology Research, Dept of Plant Sciences and Landscape Architecture, University of Maryland
Interception of fungal feeding structures by plant disease resistance proteins at the host-pathogen interface

11:35 **Jinyoung Yang Barnaby,** Department of Biology, Duke University, Durham, NC, and USDA-ARS CSGCL, Beltsville, MD
A circadian clock associated (CCA1) gene regulates the timing of the plant immune responses to downy mildew

12:00 Business meeting

12:15 **Lunch, posters, exhibits**

Session II, cont. GLOBAL REGULATION, cont.

1:40 **Vijaya Shukla** USDA-ARS Sustainable Agricultural Systems Laboratory, Beltsville, MD
Box C/D snoRNA transcription as a target of spermidine-spermine action in tomato fruit

Session III **INTRA- AND INTERCELLULAR MOVEMENT**
Moderator: **John Hammond**, USDA-ARS Floral and Nursery Plants Research Unit, Beltsville, MD

2:05 **Phillip Harries**, Department of Biology, Pittsburg State University, Pittsburg, KS
Hijacking the host cell cytoskeleton to drive virus movement in plants

2:40 **Coffee break: Poster session and Exhibitors**

3:10 Introduction of Keynote speaker: **Leslie Wanner**, USDA-ARS, Genetic Improvement of Fruit and Vegetables Laboratory, Beltsville, MD

3:20 **Patricia Zambryski ,** Department of Plant and Microbial Biology, University of California Berkeley
Making connections: Intracellular signaling regulates plant cell-to-cell transport via plasmodesmata

4:30 close of day (building closes)

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Tuesday August 16, 2011

9:00 **Registration and poster session and exhibitors**

Session IV **SYSTEMS BIOLOGY**
Moderator, **Jim Tokuhisa**, Department of Horticulture, Virginia Tech, Blacksburg, VA

9:30 **Andy Pereira**, Crop, Soil & Environmental Sciences, University of Arkansas, Fayetteville, AR and Virginia Bioinformatics Institute, Virginia Tech, Blacksburg VA
Systems biology to stress response

10:05 **John Everard**, DuPont Agricultural Biotechnology, Wilmington DE
Metabolic Engineering for Soy Quality Traits

10:40 **Coffee break: Poster session and Exhibitors**

11:00 **Dorothea Tholl**, Department of Biological Sciences, Virginia Tech, Blacksburg, VA
Elucidating the metabolism of above- and below-ground plant "volatile" defenses

11:35 **Rachel L. Melnick** USDA-ARS Sustainable Perennial Crops Lab, Beltsville, MD
The role of phytohormones in the interaction between* Theobroma cacao *and* Moniliophthora perniciosa*, causal agent of witches' broom disease

12:00 **Jianhong Chang**, Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD
***Investigating the mechanism of RTE1 regulation of the ethylene receptor ETR1 in* Arabidopsis**

12:25 **Lunch, posters, exhibits**

Session V **WHERE ARE WE GOING FROM HERE?**

Moderator, **Caren Chang**, Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD

1:30 **Frank & Kathy Turano** Plant Sensory Systems, Baltimore, MD
Moving from science to business?

2:05 **Stéphane Deschamps,** DuPont Agricultural Biotechnology, Wilmington DE
Next generation DNA sequencing applied to trait discovery and marker development in crops

2:40 **Hua Lu**, Department of Biological Sciences, University of Maryland Baltimore County, Baltimore, MD
***Multiple Roles of WIN3 in Regulating Disease Resistance, Cell Death, and Flowering Time in* Arabidopsis**

3:05 **Close of day. Thanks for your participation. Please take down your posters**

Poster #	Abstract page	**Author**(s); affiliation: ***TITLE***
01		**Richard Sicher** and Jinyoung Yang Barnaby USDA-ARS Animal and Natural Resources Institute, Beltsville, MD 20705 ***CARBON DIOXIDE ENRICHMENT MODIFIES RESPONSES OF MAIZE LEAF TRANSCRIPTS AND METABOLITES TO WATER STRESS***
02		**Kun Ji** [1, 2], Dapeng Zhang[1], Stephen Pinney[1], Sue Mischke[1], Lambert Motilal[3], and Lyndel W. Meinhardt[1] [1]USDA/ARS, Beltsville Agricultural Research Center, PSI, SPCL, 10300 Baltimore Avenue, Bldg. 001, Rm. 223, BARC-W, Beltsville, MD; [2]College of Horticulture and Landscape, Southwest University, Chongqing, 400715, China; [3]Cocoa Research Unit, The University of the West Indies, St. Augustine, Trinidad, Rep. Trinidad and Tobago, West Indies ***USING SINGLE NUCLEOTIDE POLYMORPHISM (SNP) MARKERS TO EXAMINE POPULATION STRUCTURE IN FARMER VARIETIES OF CACAO FROM HONDURAS AND NICARAGUA***
03		**Reham M. Youssef**[1,2], Margret H. MacDonald[1], Eric P. Brewer[1], Gary R. Bauchan[1], Sanaa Haroon [2], Benjamin F. Matthews[1] [1]USDA-ARS,PSI, Soybean Genomic and Improvement Laboratory, 10300 Baltimore Ave, Building 006, Beltsville, MD 20705' [2]Plant Protection Department, Faculty of Agriculture, Fayoum University, Egypt ***TRANSFERRING BASIC KNOWLEDGE FROM ARABIDOPSIS THALIANA TO THE FIELD***
04		**Michael B. McMahon**[1], Christine L. Stone[1], Melissa L. Carter[1], Laurie L. Fortis[2], Alberto Nuñez[2], Reid D. Frederick[1] and Douglas G. Luster[1] [1]USDA ARS FDWSRU, Ft. Detrick, MD; [2]USDA ARS ERRC, Wyndmoor, PA ***IDENTIFICATION OF PHAKOPSORA PACHYRHIZI APPRESSORIUM PROTEINS***
05		**Parsa Hosseini**[1,2], Arianne Tremblay[1], Nadim W. Alkharouf[2], Benjamin F. Matthews[1] [1]Towson University, Department of Computer Science, Towson, MD; [2]United States Dept. Agriculture – Soybean Genomics & Improvement Laboratory, Beltsville, MD ***DETECTING TRANSPOSONS IN PLANT PATHOGENIC FUNGI USING PROFILE HIDDEN MARKOV MODELS***
06		**Amanda Vickers**[1], Sunshine Brosi[1], James Howell[2], David P. Puthoff[1] [1]Department of Biology, Frostburg State University, Frostburg, MD; [2]Department of Biology, Allegany College of Maryland, Cumberland, MD ***QUANTIFICATION OF MEDICINAL COMPOUNDS IN VEGETATIVE AND REPRODUCTIVE ACTAEA RACEMOSA L.***
07		**Annette DeCapite,** Tyler Lancaster and David P. Puthoff Department of Biology, Frostburg State University, Frostburg, MD 21532 ***ELUCIDATION OF TERPENOID MEDICINAL COMPOUND LEVELS IN BLACK COHOSH (*ACTAEA RACEMOSA*) RHIZOMES IN RESPONSE TO PLANT DEFENSE SIGNALING MOLECULES***
08		**Annette DeCapite,** Tyler Lancaster and David P. Puthoff

Department of Biology, Frostburg State University, Frostburg, MD 21532

EXTRACTS FROM TOMATO LEAVES CARRYING HESSIAN FLY-RESPONSIVE GENES 1 AND 3 FROM WHEAT (T. AESTIVUM) AFFECT THE DEVELOPMENT OF FRUIT FLIES

09 **Ahmed M. Alzohairy**[1], Margaret H. MacDonald[2], Benjamin F. Matthews[2]
[1]Genetics Department, Faculty of Agriculture, Zagazig University, Zagazig, Egypt; [2]USDA-ARS, Soybean Genomics and Improvement Laboratory, Beltsville, MD

ISOLATION OF RESPONSIVE PROMOTERS LOCALLY INDUCED BY SOYBEAN CYST NEMATODE IN FEEDING SITES IN SOYBEAN ROOTS

10 **Ronald T. Collins**
USDA-ARS Sustainable Perennial Crops Laboratory, Beltsville, MD 20705

HOW VARIATIONS IN ANTIBIOSIS METHODOLOGY AFFECTED THE EVALUATION OF DIFFERENT TRICHODERMA ISOLATES TO CONTROL MONILIOPHTHORA PERNICIOSA (MP) AND M. RORERI (MR)

11 **David C. Henderson**[1], Basavaraj Bagewadi[2], Kael Fischer[3], Ramon L. Jordan[1], David Wang[4], Keith L. Perry[5], Ulrich Melcher[6], Claude Fauquet2, John Hammond[1]
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AN IMPROVED NUCLEIC ACID EXTRACTION METHOD AND USE FOR MICROARRAY-BASED DETECTION AND IDENTIFICATION OF VIRUSES IN PLANTS

12 **Anna Maria Vaira**[1,2], Hyoun-Sub. Lim[3,4], Robert A. Owens[4], Margaret M. Dienelt[2], Michael D. Reinsel[2], and John Hammond[2]*
[1]CNR, Istituto di Virologia Vegetale, Strada delle Cacce 73, Torino 10135, Italy; [2]USDA-ARS, USNA, FNPRU, 10300 Baltimore Avenue, Beltsville, MD; [3]Chungnam National University, Daejeon, 305-764, South Korea 20705; [4]USDA-ARS, PSI, MPPL, 10300 Baltimore Avenue, Beltsville, MD 20705

BOTH CARBOXY-COTERMINAL COAT PROTEIN FORMS OF LOLIUM LATENT VIRUS ARE REQUIRED FOR EFFICIENT SYSTEMIC MOVEMENT IN NICOTIANA BENTHAMIANA

13 **Mitchell Hall**, Vesna Atanasova, and Ed Eisenstein
University of Maryland Institute of Bioscience and Biotechnology Research, 9600 Gudelsky Dr. Rockville, MD 20850

HETEROLOGOUS EXPRESSION AND PRODUCTION OF A TOBACCO N (DISEASE RESISTANCE) PROTEIN AND ITS DOMAINS

14 **Carol D. Allen**[1], Michael D. Reinsel[2], John Hammond[2]
[1]Department of Plant Science and Landscape Architect, University of Maryland, College Park, Maryland 20742; [2]Floral and Nursery Plants Research Unit, USDA-ARS, United States National Arboretum, B-004, 10300 Baltimore Avenue, Beltsville, MD 20705

A COMPARISON OF SCREENING METHODS FOR CYMBIDIUM MOSAIC VIRUS IN ORCHIDS

15 **Jennifer Shemansky**, Christopher McClellan and Caren Chang
University of Maryland, Dept of Cell Biology and Molecular Genetics, College Park, MD

RTE3: A NOVEL PROTEIN THAT IS A MEMBER OF A NOVEL PROTEIN COMPLEX IN ARABIDOPSIS

16 **Dilip K. Lakshman**[1], John Hammond[1] and Arun K. Dhar[2]

[1]USDA-ARS, Floral & Nursery Plants Research Unit, Beltsville, Maryland 20705, USA; [2]Former affiliations: Advanced BioNutrition Corp., Columbia, MD; Viracine Therapeutics Corp., Columbia, MD

17 **Dilip K. Lakshman**[1], Ruchi Pandey[2], Kathy Kamo[3], and Gary Bauchan [4]
[1,2,3]USDA ARS, BARC, Beltsville, Maryland, [1,2]Sustainable Agricultural Research Lab and [3,4]Floral and Nursery Plants Research Unit, Maryland

AGROBACTERIUM-MEDIATED TRANSFORMATION OF* FUSARIUM OXYSPORUM *F.SP. GLADIOLI TO STUDY PATHOGENESIS IN GLADIOLUS

18 **James Tokuhisa**, William Wadlington, Chris Wolberg and Alice Mweetwa
Department of Horticulture, Virginia Tech, Blacksburg, VA

HOW DO PLANTS MAKE CYTOTOXINS WITHOUT CRIPPLING THEMSELVES? STEROIDAL GLYCOALKALOID BIOSYNTHESIS IN WILD POTATO

19 **Ahmed Ismail**[1], Benjamin F. Matthews[2] and Nadim W. Alkharouf[1]
[1]Department of Computer and Information Sciences, Towson University, Towson MD 21252 and [2]Soybean Genomics and Improvement Lab, USDA-ARS, Beltsville MD 20705

***COMPARATIVE GENOMICS ANALYSIS TO IDENTIFY LETHAL GENES IN ROOT KNOT NEMATODE (*MELOIDOGYNE *Spp) FROM* C. ELEGANS**

20 **Omar M. Darwish**[1], Lisa J. Rowland[2] and Nadim W. Alkharouf[1]
[1]Towson University, Department of Computer and Information Sciences, 8000 York Road, Towson, MD 21252; [2]USDA-ARS, Genetic Improvement of Fruits and Vegetables Laboratory, BARC-West, Beltsville, MD 20705

ANALYSIS OF BLUEBERRY 454 EST SEQUENCING DATA FROM DIFFERENT STAGES OF FRUIT DEVELOPMENT AND FLOWER BUDS AT FOUR DIFFERENT STAGES OF COLD ACCLIMATION

IDENTIFICATION OF SOYBEAN GENES INVOLVED IN RESISTANCE TO ASIAN SOYBEAN RUST USING VIRUS-INDUCED GENE SILENCING

Kerry F. Pedley[1], Ajay K. Pandey[1,2], Mandy D. Kendrick[1], Michelle A. Graham[3,4], John H. Hill[2], and Steven A. Whitham[2]

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Asian soybean rust is an aggressive foliar disease caused by the obligate biotrophic fungus *Phakopsora pachyrhizi*. Outbreaks of the disease have the potential to severely affect crop yield, and all commercially-grown cultivars of soybean are susceptible to the pathogen. Germplasm screening efforts have identified five genes (*Rpp1 – Rpp5*) that confer varying levels of resistance to select isolates of *P. pachyrhizi*. We are using virus-induced gene silencing (VIGS) in an effort to identify and characterize these resistance genes and the gene networks through which they operate. In recent years, VIGS has become an important molecular tool for studying the function of specific plant genes, including those involved in defense. A set of DNA-based VIGS vectors has been developed for use in soybean, and when combined with the genomic sequence, transcriptome analysis, gene mapping studies, and metabolomic data, VIGS is a powerful reverse genetics tool to assess functionality. To date, we have screened over 150 soybean genes and identified several that compromise resistance when silenced. An overview of the approach and a summary of genes that contribute to rust resistance will be presented.

DISCOVERY OF NEW SOYBEAN AND SOYBEAN RUST GENES USING NEXT GENERATION SEQUENCING

Arianne Tremblay[1], Parsa Hosseini[1], Shuxian Li[2], Nadim W. Alkharouf[3] and Benjamin F. Matthews[1]

[1]Soybean Genomics an d Improvement Laboratory, United States Department of Agriculture-Agricultural Research Service (USDA-ARS), Beltsville, MD 20705, U.S.A., [2]USDA-ARS, Crop Genetics Research Unit, Stoneville, MS 38776 and [3]Towson University, 8000 York Road, Towson, MD 21252

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Soybean is one of the top five agricultural products in the United States and is highly susceptible to soybean rust (SR), an exotic obligate fungus that arrived in the USA in 2004. We used mRNA-Seq by Illumina/Solexa to analyze gene expression patterns of the host and pathogen at different time points during infection of the leaf. Over 6 million sequences were obtained for each time-point. DNA sequences were aligned to the soybean genome and to SR EST sequences. Tag counts were obtained for each gene and used as a measure of expression of each gene. Then expression levels in infected leaf cells were compared to the uninfected control. Expression levels of genes encoding enzymes were overlaid on biochemical pathway diagrams from the Kyoto Encyclopedia of Genes and Genomes to visualize changes. New generation sequencing allows discovery of new genes. We found sequences aligning to portions of the soybean genome with no gene annotation. A good example of this is a contig built from 699 sequence tags aligning to chromosome 6, between base pair 48,935,656 and 48,935,919. This sequence of 263 base pairs resides within a long stretch of 14,628 base pairs on chromosome 6 where no gene has been annotated. Translation of this stretch of nucleotides revealed one open reading frame with a potential to encode five short proteins ranging from 70 to 179 amino acids. The first four proteins had high similarity to retrotransposon protein from soybean and rice while the last protein had high similarity to a disease resistance protein from *Brassica rapa*. Many other contigs did not align to the soybean genome and did not have homology to SR sequences. Sequence information on SR is limited, therefore many of these unknown sequences may be SR sequences. Some of the contigs not matching current soybean and SR databases had similarity with genes in the NCBI database including genes involved in fungal development, lignin degradation, signal transduction and intracellular communication (chitin deacetylase, glyoxal oxidase, serine threonine protein phosphatase, transthyretin). However, most of the contigs did not share similarity with genes in NCBI database but a manual homology search against different domain databases gave us numerous contigs encoding proteins containing signal peptides which are common to fungal virulence factors. We also found contigs encoding proteins containing catalase and peroxidase domains, which are involved in defense. Such information may be used to update our knowledge of the soybean and SR genomes and to develop new methods to broaden resistance of soybean to soybean rust.

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INTERCEPTION OF FUNGAL FEEDING STRUCTURES BY PLANT DISEASE RESISTANCE PROTEINS AT THE HOST-PATHOGEN INTERFACE

Wenming Wang, Robert Berkey, Xianfeng Ma, Yi Zhang, Harley King, Zhiyong Pan, Dipti Bendigeri and **Shunyuan Xiao**

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Many fungal and oomycete pathogens invade plants with a specialized infection structure named the haustorium for deriving nutrients from the host cell and cause serious diseases; in response plants have evolved elaborate defense mechanisms to fight against infection. Mechanisms of host-pathogen recognition and defense signaling have been extensively studied, however very little is known about the molecular warfare at the host-pathogen interface—the extra-haustorial membrane (EHM). We recently found that the Arabidopsis broad-spectrum resistance protein RPW8 and its family members are specifically targeted to the EHM encasing the haustorium of powdery mildew whereby RPW8 activates a defense strategy that concomitantly enhances the encasement of the haustorial complex and onsite accumulation of H_2O_2 to constrain the haustorium. To understand how RPW8 is targeted to the EHM and directs salicylic acid-dependent defense to the host-pathogen interface, a large scale mutational analysis has been conducted to identify the EHM-targeting motifs and amino acids essential for defense function in RPW8, and genetic approaches have been taken to identify novel components required for targeting RPW8 to the EHM. In addition, RPW8 has been used to investigate the origin and biogenesis of the enigmatic EHM and as a delivery vehicle to target antimicrobial cargo proteins to the host-pathogen interface. Recent research progress along these lines will be discussed.

A CIRCADIAN CLOCK ASSOCIATED (CCA1) GENE REGULATES THE TIMING OF PLANT IMMUNE RESPONSES TO DOWNY MILDEW

Jinyoung Yang Barnaby[*,1†], Wei Wang[*,1], Yasuomi Tada[1‡], Hairi Li[2], Mahmut Tor[3], Daniela Caldelari[1], Dae-un Lee[1], Xian-Dong Fu[2], and Xinnian Dong[1]

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Hyaloperonospora parasitica belongs to a group of ~ 800 fungal plant pathogens that cause Downy mildew, an economically important disease affecting numerous plant species. Resistance or (R)-gene-mediated defense is the the principal immune mechanism in higher plants providing protection against these obligate pathogens. It has been proposed that R-gene mediated defense and broad-spectrum basal defense in plants overlap. However, the underlying molecular mechanism is largely unknown. Here we report the identification of 22 novel genes involved in R-gene-mediated resistance against downy mildew in *Arabidopsis thaliana* and their regulatory control by the circadian regulator, CIRCADIAN CLOCK-ASSOCIATED 1 (CCA1). Numerical clustering analysis based on disease phenotypes of these 22 gene mutants revealed that programmed cell death (PCD) is the major contributor to disease resistance. Mutants compromised in the R-gene-mediated PCD were also defective in basal resistance, establishing an interconnection between these two distinct defense mechanisms in higher plants. Through a combination of promoter analysis and RASL-Sequencing analysis (RNA Annealing, Selection and Ligation), we showed that these newly identified defense genes are under circadian control by CCA1, allowing plants to 'anticipate' infection at dawn when the pathogen normally disperses its spores and to 'time' endogenous immune responses based on the perception of different pathogenic signals following infection. Analyzing temporal control of the defense genes by CCA1 allowed us to differentiate their involvement in basal and R-gene-mediated defense. Our study has, for the first time, revealed that a plant's defense system cycles on a daily basis, even when pathogens are absent, and provided 22 defense candidate genes for engineering plant resistance against downy mildew with obvious potential benefits to hundreds of agricultural and horticultural plant species.

(Published in Nature 470, 110–114 (03 February 2011) doi:10.1038/nature09766)

BOX C/D SNORNA TRANSCRIPTION AS A TARGET OF SPERMIDINE-SPERMINE ACTION IN TOMATO FRUIT

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USDA-ARS Sustainable Agricultural Systems Laboratory, Beltsville Agricultural Research Center, MD 20705

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Molecular genetics approaches have added credence to the previous physiological and some hypothetical studies on the role(s) of polyamines in plants. Our laboratory uses transgenic approaches with tomato fruit as a gain-of-function model to study effects of polyamines on cellular metabolism, nutritional quality, antioxidant capacity, fruit texture, vine and shelf life, disease and decay symptoms. Fruit-specific expression of yeast S-adenosylmethionine decarboxylase in tomatoes led to high accumulation of polyamines spermidine (Spd) and spermine (Spm) at the cost of putrescine (Mehta et al. 2002). Subsequent transcriptional (Srivastava et al. 2007) and metabolomic (Neelam et al. 2008) studies indicated that Spd and Spm act as anabolic growth regulators in tomato. Several ribosomal RNA protein genes including 60S protein L4, 50S protein L15, and 40S protein S9 as well as a number of chaperone genes and many stress responsive transcription factors predominated in high Spd-Spm accumulating fruits. We hypothesized that these biogenic amines positively impact protein synthesis by influencing biogenesis, transcription, processing and stability of ribosomal RNA (rRNA) genes, and subsequently *de novo* protein synthesis in tomato fruit. One major biogenetic process regulating transcription and processing of pre-mRNA complexes in the nucleus involves the role of small nucleolar RNAs (snoRNAs). Therefore, we cloned, sequenced and identified a box C/D snoRNA cluster in tomato. The snoRNA cluster comprises *SlSnoR12.1* and *SlU24* separated by an 'AT' rich spacer and carries conserved structural elements such as internal (C & D) and external (C' & D') boxes. Molecular characterization showed these snoRNAs to be transcribed di-cistronically. Upstream sequence of *SlsnoR12.1* carries putative 'TATA', homol E and D box-like sequences. Interestingly, the snoRNA cluster was found to harbor at their 5' and 3' flanking region several *cis* elements, particularly ERE, responsive to the fruit ripening hormone ethylene. Quantitative PCR (Q-PCR) analysis revealed that expression of *SlSnoR12.1* and *SlU24* declines several-fold during fruit ripening. We are now conducting Q-PCR analysis of snoRNA transcripts and certain rRNA genes in the high Spd-Spm transgenic fruit (and the azygous control) during fruit ripening to characterize the role of Spd-Spm on snoRNA transcription.

References:

Mehta et al., (2002). *Nature Biotechnology*, 20, 613–618.
Srivastava et al., (2007) *Plant Biotechnology* 24, 57–70.
Neelam et al., (2008). *Journal of Experimental Botany*, 59:2337-2344.

HIJACKING THE HOST CELL CYTOSKELETON TO DRIVE VIRUS MOVEMENT IN PLANTS

Phillip Harries[1*], Richard Nelson[2], and Brendan Coulter[1]

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As obligate parasites, viruses depend upon host cell factors for their replication and movement. Both the microtubule and actin microfilament networks have been implicated in the movement of plant viruses. Utilizing the microfilament inhibitor, Latrunculin B (LatB), we demonstrate that a number of viruses from diverse genera including *Tobacco mosaic virus* (TMV), *Potato virus X* (PVX), and *Tomato bushy stunt virus* (TBSV) are dependent upon the actin cytoskeleton for their intercellular spread. Surprisingly we found that Turnip vein clearing virus (TVCV), a close relative of TMV, did not require intact microfilaments for movement. The finding that numerous viruses rely upon microfilaments for their intercellular movement led us to investigate the role of myosin motor proteins in this process. We show that virus-induced gene silencing of an *N. benthamiana* myosin XI gene has an inhibitory effect on the cell-to-cell movement of TMV. This effect is specific to TMV since the movement of other viruses whose intercellular trafficking was dependent upon actin was not inhibited by silencing of this myosin. In addition to their reliance upon host proteins such as the cytoskeleton for movement, most plant viruses encode one or more movement proteins (MP) that increase the size exclusion limit (SEL) of the plasmodesmata (PD) and are believed to escort viral components from one cell to the next. Based on our finding that TBSV requires microfilaments for movement, we wanted to explore the possibility that TBSV MP might interact with the cytoskeleton. To this end, we have created fluorescent fusions of the TBSV MP with red fluorescent protein (RFP) in order to examine its' subcellular localization with regard to the cytoskeleton. Upon ectopic expression of TBSV MP in *Nicotiana benthamiana* cells, we observe a strong co-localization between TBSV MP and microfilaments. Interestingly, this interaction results in a dramatic disruption of the microfilament structure. We discuss the potential significance of this interaction in terms of TBSV intra/intercellular movement.

MAKING CONNECTIONS: INTRACELLULAR SIGNALING REGULATES PLANT CELL-TO-CELL TRANSPORT VIA PLASMODESMATA

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Plant cells are bound by cellulosic cell walls, creating a challenge to resource sharing and information exchange between individual cells. To overcome this, plants have evolved channels, called plasmodesmata (PD) that provide cytoplasmic continuity between each cell and its immediate neighbors. The regulation of PD formation and function impacts all stages of plant growth and development (reviewed in (1)). We use Arabidopsis embryogenesis as a model system to study PD. To identify genes that regulate PD structure/function we screened for mutants with altered cell-to-cell transport during the mid-torpedo stage of embryogenesis. We identified several mutants called *increased size exclusion limit* (*ise*), and two mutants *ise1* and *ise2* have been studied in detail. Besides altering PD function, *ise1* and *ise2* both direct the de novo formation of PD (2). *ISE1* encodes a DEAD-box RNA helicase that localizes to mitochondria (3), and *ISE2* encodes a DEVH-type RNA helicase that localizes to chloroplasts (4,5). Surprisingly, plastid development is defective in both *ise1* and *ise2* mutant embryos, and following gene silencing of *ISE1* or *ISE2* in mature tissues (5). How can two mutants that encode products that localize to different cellular compartments, both exhibit the same strong effect on PD formation and function? We performed gene expression analyses comparing *ise1* and *ise2* mutant to wild-type embryos (5). The data reveal that both mutants have dramatic effects on genes involved in chloroplast function. Of genes with assigned function whose expression is commonly altered in *ise1* and *ise2* mutants, one in five is directly involved in plastid function. Thus, their similar phenotypes may be explained by their common impact on plastid development. Further, several genes with roles in cell wall synthesis and modification are also differentially expressed in both mutants, providing new targets for investigating PD development and function. Independent analyses show that the redox state in organelles differentially regulates PD function (6). For example, when the chloroplast is in a reduced state, cell-to-cell transport increases, while when the chloroplast is in an oxidized state, cell-to-cell transport decreases. These recent findings begin to illuminate the elaborately sensitive signaling processes that plants have evolved to precisely regulate plant intercellular transport. While genetics and genomic approaches have searched for novel factors that might constitute PD structural or regulatory components, the new data reveal that plant cell homeostasis and constitutive signaling pathways provide unexpected answers to understanding the underpinnings of PD.

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DROUGHT STRESS RESISTANCE REGULATORY NETWORKS INTERACTING WITH BASIC BIOLOGICAL PROCESSES

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Plants respond to drought stresses by the activation of cascades of molecular networks involved in stress perception, signal transduction, and the expression of specific stress related genes and metabolites. To dissect the complex drought stress response pathways we analyzed gene expression changes in drought treated *Arabidopsis* and rice vegetative and reproductive tissue, revealing a high correspondence of differentially regulated orthologous genes, supporting that perception and resistance to dehydration/drought has evolved in plants through some common conserved mechanisms. To develop a systems view of drought stress response in relation to plant biological processes, we created a rice environment coexpression network (RECoN) to model the correlation between stress responsive gene expression, drought resistance and plant biological processes. Knockout mutants of 200 Arabidopsis drought stress-responsive regulatory genes (SRGs) were screened to characterize their altered growth under drought (*gud*) mutant phenotypes. Representative *gud* mutants were analyzed for changes in gene expression responses that were mapped onto the drought gene interaction networks and revealed drought-regulated genes involved in stress protective mechanisms. Overexpression analysis of a rice candidate drought SRG named HYR, revealed enhanced drought resistance and water use efficiency phenotypes associated with increased photosynthesis and grain yield, supported by gene expression changes of gene modules involved in protective biological processes. These stress response networks are useful to study the interaction of environmental stresses with plant signaling pathways and the adverse effect on important biological processes, as well as the adaptive protective mechanisms providing stress tolerance or resistance.

METABOLIC ENGINEERING FOR SOY QUALITY TRAITS

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The application of biotechnology to crop production falls into two general categories, Input and Output traits. Input traits, those that confer benefits to farmers by providing herbicide tolerance or resistance to insect pests have been rapidly adopted by the industry and over 93% of the soybean and 86% of the corn grown in the US in 2010 carried at least one transgenic Input trait. Output Traits, those designed to modify the quality and functionality of the harvested component of the plant have been slower to come to market but represent some of the best examples of value adding metabolic engineering. The portfolio of Output Traits currently under development has great potential to add value and utility to agricultural products in the near and long term. In this talk I will illustrate the soybean Output (Quality) Trait research and development program at DuPont through three examples.

1/. Improving functionality: Plenish™ High Oleic soybean oil, the first biotech soy product with direct benefit to consumers and end users, has superior functional properties when compared to conventional soybean oils and it is a strong contender as a trans-fat free alternative for frying applications. High oleic oils also have great potential in industrial applications.

2/. Increasing value: Soybeans are grown principally for their protein but the oil is a valuable co-product. Expression of heterologous diacylglycerol acyltransferases (DGATs) in soy leads to increases in oil content while maintaining protein levels, characteristics that have great potential for increasing the value that can be derived from the seed.

3/. Improving nutritional value: raffinose and stachyose comprise approximately 40% of soybean seed soluble sugars at maturity. These sugars are not digestible by mono-gastric animals (including humans) and represent an anti-nutritional factor. Using metabolic engineering we have been able to lower raffinose and stachyose levels by an order of magnitude. The utility of these modified seeds in animal rations is currently under investigation.

Through these examples I will demonstrate that metabolic engineering can be used to enhance the utility and value of agricultural products with the added benefit of providing fundamental insights into plant metabolism.

ELUCIDATING THE METABOLISM OF ABOVE- AND BELOW-GROUND PLANT "VOLATILE" DEFENSES

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Plants have evolved sophisticated defense mechanisms by emitting volatile chemicals at short and long distance. Volatile organic compounds are thought to serve multiple defensive roles such as antimicrobial or antifeedant activities or the attraction of natural enemies of insect herbivores. Because of this multifunctionality, volatile-based chemical defenses are of interest in developing and improving natural, more sustainable pest and pathogen control practices. We conduct comparative studies of the metabolism and function of volatile compounds in above and belowground tissues of *Arabidopsis* with a focus on volatile terpenes, the largest class of plant volatile metabolites. I will present recent findings on the defensive role of floral volatiles and on the biosynthesis and regulation of irregular homoterpenes (C_4-norterpenes), which are among the most common, herbivore and pathogen induced volatile compounds in leaves. We have found these volatiles to be produced in aerial and belowground tissues but by completely different pathways indicating plasticity in homoterpene formation between shoots and roots. While homoterpenes synthesized in leaves are largely implicated in anti-herbivore defense, our studies indicate that homoterpenes emitted by roots aid in the defense against microbial pathogens such as the oomycete *Pythium irregulare*. In addition to these results, I will discuss recent findings on the cell type-specific organization and subcellular localization of other terpene producing enzymes in *Arabidopsis* roots.

THE ROLE OF PHYTOHORMONES IN THE INTERACTION BETWEEN *THEOBROMA CACAO* AND *MONILIOPHTHORA PERNICIOSA*, CAUSAL AGENT OF WITCHES' BROOM DISEASE

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The agaric basidiomycete *Moniliophthora perniciosa*, causal agents of witches' broom of *Theobroma cacao* (cacao), causes severe yield losses throughout South America. Although the symptoms of *M. perniciosa* infection are typified by formation of brooms in branch tissue, infection of floral cushions can cause the development of parthenocarpic fruit commonly known as "cherimoya-like fruit" due to the distorted shape. Research suggests that hormones, produced both by cacao and *M. perniciosa,* are involved in the formation of brooms, yet the role of phytohormones in *M. perniciosa* infected fruit remains unknown. Young healthy fruits and infected fruits were obtained from three cacao clones in Bahia Brazil. RNA was extracted and QPCR analysis was conducted to determine the expression of ESTs related to phytohormone production and action in both cacao and *M. perniciosa*. Of the cacao ESTs tested, 27% were upregulated by *M. perniciosa*, 12% were downregulated by *M. perniciosa,* and the expression of the remaining 61% not being impacted by the presence or absence of *M. perniciosa.* No cacao ESTs involved in the auxin or cytokinin pathways were downregulated in parthenocarpic pods. *M. perniciosa* ESTs for the GA and IAA pathways were not upregulated during the infection process, with the exception of *MpDOPA*. The research presented will discuss the role of *M. perniciosa* in altering cacao phytohormones to initiate the development of parthenocarpic fruits.

INVESTIGATING THE MECHANISM OF RTE1 REGULATION OF THE ETHYLENE RECEPTOR ETR1 IN *ARABIDOPSIS*

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The plant ethylene hormone plays a vital role in regulating plant growth and development as well as plant defense to biotic and abiotic stresses during the entire life of the plant. In *Arabidopsis*, ethylene is perceived by a family of five receptors, one of which is ETR1. The *Arabidopsis REVERSION-TO-ETHYLENE SENSITIVITY1* (*RTE1*) gene is a positive regulator of *ETR1* that was identified in a genetic screen for suppressors of the dominant ethylene-insensitive mutation *etr1-2*. *RTE1* encodes a novel integral membrane protein and interacts with ETR1 at the Golgi apparatus and the endoplasmic reticulum (ER), but the molecular mechanism by which RTE1 regulates ETR1 remains unknown. We propose that RTE1 may affect the conformation of the ETR1 ethylene binding domain, resulting in the promotion or stabilization of the ETR1 signaling "on" state. We are investigating the molecular function of RTE1 and the basis for its specificity for regulating the ETR1 receptor isoform, and not the four other ethylene receptors.

In a library screen for RTE1-interacting proteins using the yeast split-ubiquitin assay, we identified an ER-localized cytochrome *b*5 isoform (AtCb5-D). Cb5 is a small hemoprotein that functions in oxidation/reduction reactions. Functional parallels between *AtCb5-D* and *RTE1* provide evidence for their interaction *in planta*, e.g., *Atcb5-d* loss-of-function suppresses *etr1-2* ethylene insensitivity and appears to be specific to the ETR1 receptor, while overexpression of *AtCb5-D* confers reduced ethylene sensitivity. We also found that loss-of-function mutants of two other ER-localized forms of Cb5 (AtCb5-B and AtCb5-C) can suppress *etr1-2*. Although each single *cb5* loss-of-function mutant has no ethylene-response phenotype, double *cb5* loss-of-function mutants display an enhanced ethylene response. Additional genetic analyses suggest that the *AtCb5*s might act upstream of *RTE1*. Moreover, using a hemin-agarose affinity chromatography assay, we found that RTE1 homologs are able to bind heme. Finally, we found that the basis for the specificity of RTE1 for ETR1 is due to a unique proline (P9) conserved only in ETR1 orthologs; P9 is located just three amino acids away from two cysteine residues (C4 and C6) that form disulfide bonds in the ETR1 homodimer. Taken together, these findings suggest that RTE1 may promote the active conformation of the ETR1 receptor through oxidative folding, involving AtCb5s and redox reactions.

MOVING FROM SCIENCE TO BUSINESS?

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After spending 40 years (combined) in government and academia we started an agricultural biotechnology company. In our talk we will share lessons we learned in our transition as scientists to entrepreneurs. We will present five basic questions derived from The Entrepreneur's Guide to a Biotech Startup (P. Kolchinsky) that can help in the decision of whether to turn a scientific concept into a business: do you have a viable product, is your intellectual property (IP) defensible, do you have a strategy for making a profit, is there a large market for your product, and do you have a management team that is capable of implementing the strategy? We will provide an overview of the many resources that are available to entrepreneurs and biotech start-up companies from the Internet, federal government, and state of Maryland. We will also discuss different options available to biotech start-up companies with regards to protecting IP, financing the business, and deciding where to set up "shop". Finally we will share key advice we received from veteran entrepreneurs and the path that we chose to start our company.

NEXT GENERATION DNA SEQUENCING APPLIED TO TRAIT DISCOVERY & MARKER DEVELOPMENT IN CROPS

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A majority of current molecular technologies applied to crop improvement are based on detecting DNA sequence variants, such as single nucleotide polymorphisms (SNPs), to develop markers associated with a particular trait of interest. The advent of next-generation DNA sequencing technologies has greatly accelerated the pace of DNA marker-based research, as it now enables the rapid and cost-effective characterization of complex genomes and the discovery in a matter of days of large numbers of variants for DNA marker development. DuPont is actively engaged in using these technologies for elucidating structural and functional characteristics of genes and genomes in various crop species. The talk will describe various aspects of using the Illumina next-generation DNA sequencing technology to discover SNPs in low-complexity regions of crop genomes and their use as resources for developing informative markers. The development of a genotyping-by-sequencing pipeline in crops also will be discussed.

MULTIPLE ROLES OF WIN3 IN REGULATING DISEASE RESISTANCE, CELL DEATH, AND FLOWERING TIME IN *ARABIDOPSIS*

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The salicylic acid (SA) regulatory gene *HOPW1-1-INTERACTING 3 (WIN3)* was previously shown to confer resistance to the biotrophic pathogen *Pseudomonas syringae*. Here we report that *WIN3* controls broad-spectrum disease resistance to the necrotrophic pathogen *Botrytis cinerae* and contributes to basal defense induced by flg22, a 22-amino acid peptide derived from the conserved region of bacterial flagellin proteins. Genetic analysis indicates that *WIN3* acts additively with several known SA regulators, including *PHYTOALEXIN DEFICIENT 4, NONEXPRESSOR OF PR GENES 1 (NPR1)*, and *SA INDUCTION-DEFICIENT 2* in regulating SA accumulation, cell death, and/or disease resistance in the Arabidopsis mutant *acd6-1*. Interestingly, expression of *WIN3* is also dependent on these SA regulators and can be activated by cell death, suggesting that *WIN3*-mediated signaling is interconnected with those derived from other SA regulators and cell death. Surprisingly, we found that *WIN3* and *NPR1* synergistically affect flowering time via influencing the expression of flowering regulatory genes *FLOWERING LOCUS C* and *FLOWERING LOCUS T*. Taken together, our data reveal that *WIN3* represents a novel node in the SA signaling networks to regulate plant defense and flowering time. They also highlight that plant innate immunity and development are closely connected processes, precise regulation of which should be important for the fitness of plants.

01 CARBON DIOXIDE ENRICHMENT MODIFIES RESPONSES OF MAIZE LEAF TRANSCRIPTS AND METABOLITES TO WATER STRESS

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Maize (*Zea mays* L.) was grown in indoor environmental chambers with ambient (38 Pa) and elevated (70 Pa) CO_2. Drought treatments were imposed 17 days after sowing by withholding nutrient solution. Decreases of soil water content, leaf water potential, net CO_2 assimilation and stomatal conductance due to water stress were delayed ~2 days by CO_2 enrichment. Inorganic nitrate decreased with leaf age in both CO_2 treatments. Concentrations of 28 of 33 (85%) leaf compounds involved in primary metabolism were altered by water deficiency. Soluble carbohydrates, aconitate, shikimate, serine, glycine, proline and eight other amino acids increased and leaf starch, malate, fumarate, 2-oxoglutarate and seven amino acids decreased with drought. These changes were typically delayed or diminished by CO_2 enrichment. Drought dependent decreases of nitrate, alanine and aspartate were unaffected by CO_2 enrichment and were probably influenced by limiting nitrogen. The induction of fourteen stress related maize genes to drought was delayed or modified by CO_2 enrichment and four transcripts expressed during the first week of water stress were not induced in the elevated CO_2 treatment. Metabolite changes indicated that water stress inhibited C4 photosynthesis, affected N availability, increased osmolyte concentrations, increased levels of stress related metabolites and induced photorespiration. The above results showed that water stress had profound effects on maize metabolism and that CO_2 enrichment modified responses of maize metabolites and transcripts to moderate drought but was less effective when drought was severe.

02 USING SINGLE NUCLEOTIDE POLYMORPHISM (SNP) MARKERS TO EXAMINE POPULATION STRUCTURE IN FARMER VARIETIES OF CACAO FROM HONDURAS AND NICARAGUA

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Cacao (*Theobroma cacao* L.) is the main source for chocolate with an annual production of four million tons worldwide. This neotropical tree crop was domesticated in Mesoamerica as far back as 3,000 years ago. Traditional cacao varieties suitable for the production of fine flavored chocolate still exist in farmers' fields in Mesoamerica. But, these varieties are thinly scattered among ordinary bulk varieties and are being replaced by introduced exotic varieties. Knowledge of genetic identity and population structure in farmer varieties of cacao in the center of domestication is essential for sustainable production of fine flavored cacao beans and contributes to in situ/on-farm conservation of these premium varieties. Using high throughput SNP genotyping, we analyzed 85 farmer selections varieties collected from traditional farms in Honduras and Nicaragua. A total of 32 accessions with known population membership in the International Cocoa Gene banks were used as references. Ancient Criollo and Amelonado varieties were successfully identified based on their genetic affiliation with reference varieties. Bayesian assignment test revealed that the Honduras and Nicaragua farmer varieties differ in their ancestry admixture, suggesting the multiple origins of traditional varieties in Mesoamerica. The study shows that high through-put genotyping using a small set of SNP markers, combined with model based statistical analysis, is a cost-effective and accurate approach to dissect population structure in farmer varieties. Further evaluation and use of the elite farmer varieties is an economically viable approach to conserve these fine flavored varieties.

03 TRANSFERRING BASIC KNOWLEDGE FROM *ARABIDOPSIS THALIANA* TO THE FIELD

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Soybean cyst nematode (SCN), an obligate parasite of plants, is the most damaging pathogen of soybean, causing $469 to $818 million in soybean yield losses annually in the United States. However, there are no soybean cultivars available that are resistant to all SCN populations. To achieve this goal, we cloned Arabidopsis genes proven to be important to the defense response and over expressed them in soybean. For example, a large number of genes involved in jasmonic acid (JA) and salicylic acid (SA) signaling, production and response play a crucial role in basal and R gene mediated resistance to biotrophic pathogens. Mutant plants compromised in JA and SA biosynthesis and signaling exhibit decreased resistance to biotrophic pathogens and fails to trigger the expression of PR genes in response to pathogen attack. In this work, we over expressed an *Arabidopsis thaliana* Gene A in soybean roots using PRAP15 as vector. This vector was designed to express enhanced green fluorescent protein (eGFP) so that transformed roots could be recognized easily. We tested this vector by insertion of the gene encoding Red Fluorescent Protein (RFP) into the cloning site and transforming onion cells using the biolistic

methods. The Gene A construct was transformed into soybean roots using an *Agrobacterium rhizogenes* based transformation system. Transformed roots were challenged with SCN and root-knot nematode (RKN). The numbers of the mature SCN females were counted after 35 days after inoculation and numbers of galls formed by RKN were counted after 30 days after inoculation. Reduction in the number of the nematodes will indicate that Gene A may be useful for engineering resistance in soybean to SCN.

04 IDENTIFICATION OF PHAKOPSORA PACHYRHIZI APPRESSORIUM PROTEINS

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Phakopsora pachyrhizi, which causes Asian soybean rust (ASR), has spread from southern Asia and Australia to Africa and South America, and more recently to North America. At present, U.S. commercial soybean cultivars do not have resistance to ASR. To develop novel methods for controlling this disease, it is important to understand the molecular and biochemical processes that occur throughout the infection cycle. A critical step in the infection process is the formation of appressoria, by which *P. pachyrhizi* directly penetrates the host surface. This study identified proteins present during appressorium formation. A 2-D gel and MS approach was applied to identify 256 proteins from a custom database comprised of 1040 ESTs from a *P. pachyrhizi* appressorium-enriched cDNA library and Basidiomycota EST sequences from NCBI. Fifty-nine of these proteins were not previously identified in a partial proteome of *P. pachyrhizi* germinating urediniospores. Proteins with roles in primary metabolism and energy accounted for 25% of the 59 selected proteins and hypothetical proteins with unknown function made up 24%. Eight of the 14 unknown proteins had signal peptides, and three are putative extracellular proteins. This information will be useful in future research on protein expression during early stages of the infection process.

05 DETECTING TRANSPOSONS IN PLANT PATHOGENIC FUNGI USING PROFILE HIDDEN MARKOV MODELS

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Currently, very little is known about transposable elements in plant-pathogenic fungi. Otherwise known as 'jumping genes', transposons can mediate biological changes such as sequence variation and modification of gene activity. Recent studies have shown that such elements have given rise to numerous gene promoters, genes, and miRNAs.
We present a probabilistic model for the prediction of transposable elements in plant-pathogenic fungi. Data and literature mining retrieved a set of 35 biotrophic plant fungal transposons, ranging from *Agaricus bisporus* to *Tolypocladium inflatum*. Homology and sequence analysis was performed for each pathogen, resulting in a multiple-sequence 'profile' model. This profile model, made using HMMER homology sequence tool and custom Python scripts, represents conserved regions in the multiple sequence alignment and probabilistic states for each nucleotide. Given a query sequence, calculations can be made as to how probable motifs within this sequence compare to that of the 35 biotrophic plant pathogens. Research in plant-pathogenic fungal transposons is in its infancy with a major roadblock being the fact that genomes of many pathogens are still being sequenced or yet to be annotated. However, with publicly available resources, our probabilistic models and analysis of known fungal transposon sequence-families can hopefully help shed light on projects dealing with evolutionary variation and gene expression.

06 QUANTIFICATION OF MEDICINAL COMPOUNDS IN VEGETATIVE AND REPRODUCTIVE *ACTAEA RACEMOSA* L.

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Black cohosh (*Actaea racemosa* L.) is a perennial herb native to deciduous woodlands in Eastern North America used traditionally as a specific remedy for musculoskeletal pain and to support uterine function. Today, extracts of black cohosh are marketed primarily for use in relieving hot flashes with a majority of the raw material supplying this global market being harvested from naturally occurring wild populations in Appalachian woodlands. In Maryland and throughout the Appalachians, a vegetatively similar but vulnerable species (IL S1, KY S5, MD S2, NC S4, PA S3, WV S3) species, mountain bugbane (*A. podocarpa* DC.), is concurrent and at risk by accidental collection due to confusion with *A. racemosa*. This research addresses whether harvesting only individuals with distinctive reproductive seeds would address both issues of authentication and conservation. Selecting only reproductive individuals would allow a wild harvester to ensure botanical identity and potentially increase the yield of medicinal compounds. This would provide an economic incentive and simultaneously protect threatened species. In August 2010, 40 plants were harvested from naturally occurring populations in

western Maryland. Reproductive plants failed to differ in rhizome mass from neighboring vegetative counterparts ($p = 0.429$), indicating similar maturity. All rhizomes were extracted and quantified for actein and 23-epi-26 deoxyactein using HPLC. Mann-Whitney rank sum test comparing median concentrations of deoxyactein and actein showed significant differences for deoxyactein between reproductive (M=1.304 mg/g rhizome) and non-reproductive (M=1.261 mg/g rhizome) plants at the <0.1 level ($p = 0.090$). No significant difference in actein levels were detected between reproductive and non-reproductive individuals. Future research will explore these differences across broader geographic scales.

07 ELUCIDATION OF TERPENOID MEDICINAL COMPOUND LEVELS IN BLACK COHOSH (*ACTAEA RACEMOSA*) RHIZOMES IN RESPONSE TO PLANT DEFENSE SIGNALING MOLECULES

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Actaea racemosa (Black Cohosh) is native to eastern North America and found most often in woodland openings. Black Cohosh is most often used as a treatment for menopausal symptoms and has potential to reduce the risk of breast cancer. Black Cohosh supplements accounts for nearly $9.7 million of the U.S dietary supplement and herbal sales. Traditionally, *A. racemosa* rhizomes and roots are the part of the plant which is ground into the herbal supplement. The secondary metabolites, actein and deoxyactein are two compounds (among many others) associated with activity and are many times used for standardization of supplements. However, due to the nature of the *A. racemosa*, it is typical that only a small quantity of active ingredients is produced in each rhizome. These low yields cause many plants to be harvested from wild populations as Black Cohosh cultivation is not always profitable. Increasing the yield of actein and deoxyactein would help conserve wild populations that are relatively unstudied and may be eliminated because of harvesting. Secondary metabolites, such as actein and deoxyactein, are triterpenes and are typically synthesized as a defense mechanism of the plant. In times of predator attack the production of secondary metabolites are increased in an attempt to prevent continued predation. In order to gain an understanding of what defensive pathways are involved in actein and deoxyactein synthesis, plant defense signaling compounds will be used to try to stimulate actein and deoxyactein production. These include jasmonic acid, salicylic acid, ethylene, and plant wounding. With the determination of signaling molecules that can stimulate the production of actein and deoxyactein, the function of actein and deoxyactein can begin to be elucidated. To assess the levels of actein and deoxyactein within rhizomes, HPTLC will be used.

08 EXTRACTS FROM TOMATO LEAVES CARRYING HESSIAN FLY-RESPONSIVE GENES 1 AND 3 FROM WHEAT (*T. AESTIVUM*) AFFECT THE DEVELOPMENT OF FRUIT FLIES

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Billions of dollars are lost each year worldwide due to insect damage of crops and the resulting food. Although many strategies exist for improving crops and limiting insect damage, the identification and incorporation of genes from many species has advantages. Three genes from wheat, Hessian fly responsive – 1, -2 and -3, with the potential to inhibit insect growth were transformed into tomato using *Agrobacterium tumefaciens* methodology. Plants carrying one of the three genes were selected using kanamycin and the presence of the transgene was confirmed with PCR. Leaf tissue from multiple transgenic lines carrying one of the three *Hfr* genes was used to create aqueous extracts. These extracts were then incorporated into artificial Drosophila growth media. The media was inoculated with flies and the resulting insect progeny were monitored for 21 days for pupation and eclosion. Extracts from Hfr-1 and Hfr-3 plants significantly delayed the development of larvae. Plants were also used as food source for tobacco hornworm larvae but did not show an inhibitory effect. The identification of genes and their products useful in protecting plants from damage and herbivory will enable the production of the additional food needed to feed a growing world.

09 ISOLATION OF RESPONSIVE PROMOTERS LOCALLY INDUCED BY SOYBEAN CYST NEMATODE IN FEEDING SITES IN SOYBEAN ROOTS

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For successful engineering of transgenic plants for nematode resistance, it is important to regulate plant resistance with promoters that over-express resistance genes or produce RNAi gene silencing in the correct cells at the proper time. Our previous microarray experiments data of laser-captured, micro-dissected samples showed a list of soybean (*Glycine max*) genes that are responsive and activated upon soybean cyst nematode (*Heterodera glycines*) infection after 3, 6 and 9 days at the feeding site in the resistant cultivar Peking. In this investigation, we have identified, isolated, cloned seven different promoter regions, harboring different regulatory elements upstream of genes that are highly induced by nematode infection in all the studied time points and expressed in nematodes feeding sites from the susceptible cultivar, Williams82

The upstream cloned promoters were sequenced and transferred to the expression vectors pGWB404, which drives green fluorescent protein, and pKGWFS7, which drives beta-glucuronidase (GUS) expression in transformed roots.
Both promoter-testing Gateway vector constructs were transferred to onion cells using biolistic gene transfer to confirm their activity in plant tissue. These promoter constructs are currently cloned into *Agrobacterium tumefaciens* strain GV3101to transform Arabidopsis by floral dip method, and also cloned in *Agrobacterium rhizogenes* strain K599 to transform soybean. Transformed plants will be assayed for their local expression during nematode infection. These promoters will provide scientists with useful regulators for expressing genes and gene products that may inhibit the life cycle of the soybean cyst nematode specifically at nematode feeding site.

10 HOW VARIATIONS IN ANTIBIOSIS METHODOLOGY AFFECTED THE EVALUATION OF DIFFERENT *TRICHODERMA* ISOLATES TO CONTROL *MONILIOPHTHORA PERNICIOSA* (MP) AND *M. RORERI* (MR)

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Some beneficial fungus produces compounds which are inhibitory to pathogenic fungal growth. These compounds can be broken down into three broad categories: enzymatic, volatile, and not enzymatic-not volatile. The cost and time required to evaluate all three categories of antibiosis compounds for a large number of isolates is prohibitive. Consequently, not enzymatic-not volatile is the only evaluation conducted, because the others are considered minimal.

The initial step in preparation for the antibiosis testing, after the beneficial fungus has grown in a liquid media, is the removal of all fungal material from the media. This can be accomplished by either syringe filtration or centrifugation. The determining factor is whether a suitable centrifuge is present in the laboratory.

The purpose of this study was twofold. First, determine if the antibiosis results differed depending on rather syringe filtration or centrifugation was used. Second, determine if the volatile and enzymatic compounds activity had a major effect.

Representative samples of isolates were selected from the populations of isolates being evaluated. The samples were prepared with syringe filters and centrifugation. After the samples were prepared the isolates were evaluated for all three categories. First, it was found that centrifuged and syringe filter preparation were equivalent. Second, there was no difference between antibiosis test for enzymatic, volatile and not volatile-not enzymatic compounds for MR; enzymatic compounds were significantly different for MP.

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11 AN IMPROVED NUCLEIC ACID EXTRACTION METHOD AND USE FOR MICROARRAY-BASED DETECTION AND IDENTIFICATION OF VIRUSES IN PLANTS

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Globalization has increased the risk that pernicious plant pathogens will be introduced or reintroduced into susceptible plant populations. Because imported plants and germplasm harboring infections may not present obvious disease symptoms, a rapid assay that can detect known and even uncharacterized viruses at low titer is required. To address this concern we are developing a microarray-based assay for the identification and categorization of plant viruses to at least the genus level. Many plant species produce a variety of metabolites that impair timely isolation of pure nucleic acids. Isolation methods often are designed for a limited subset of species and are not widely tested. We have incorporated KOAc and spin columns into a CTAB-based protocol for isolating an acceptable yield of amplifiable total nucleic acid (TNA) from a wide variety of plants in about two hours. The CKC method has been tested successfully on over 60 plant species from 35 diverse plant families, including many species known to contain significant levels of polysaccharides, phenolics, alkaloids, and resins; RNA quality was measured and determined to be suitable to generate reliable real-time PCR results. To probe isolated nucleic acids for the presence of viral genomes, we have designed and produced a 10,000 element Universal Plant Virus Microarray (UVPM) composed of 60-mer oligos covering every taxon/node of the taxonomic tree for all plant viruses available in GenBank. Currently, we are building a database of array signatures from known viruses. Because some viruses may be present at very low titer in plant tissue, we are developing amplification methods to avoid misdiagnoses and false-negative assay results.

12 BOTH CARBOXY-COTERMINAL COAT PROTEIN FORMS OF *LOLIUM LATENT VIRUS* ARE REQUIRED FOR EFFICIENT SYSTEMIC MOVEMENT IN *NICOTIANA BENTHAMIANA*

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The *Lolium latent virus* (LoLV; genus *Lolavirus*, family *Alphaflexiviridae*) genome is encapsidated by equimolar amounts of carboxy-coterminal coat protein (CP) variants of apparent MW 33 and 28kDa. The CP ORF contains two 5'-proximal AUGs, encoding Met 1 and Met 49, respectively promoting translation of the 33kDa and 28kDa CP variants. The 33kDa CP N-terminal domain includes a 42 aa sequence encoding a putative chloroplast Transit Peptide (cTP) with a predicted cleavage site upstream of AUG2. Ablation of AUG1 in an infectious clone yielded mutant LoLV-K1, which was able to replicate in inoculated leaves of *Nicotiana benthamiana*, but not spread systemically. Mutation of AUG2 to UUG yielded mutant LoLV-K2, which was able to infect plants systemically. LoLV-K1 revertants that regained expression of a CP form of >28kDa (by restoration of wild-type AUG1; by mutation to AUG at another site; or by mutation to an upstream CUG alternate initiation codon) were able to infect plants systemically. Mutation of AUG2 to CCC prevented possible initiation at UUG, but still allowing potential upstream proteolytic cleavage of the cTP region to form c.28kDa CP. Substitution of four amino acids at the predicted cTP cleavage site combined with AUG2>CCC yielded mutant LoLV-C4, in which systemic infection was significantly delayed and symptoms altered. The N-terminal cTP sequence is crucial for efficient cell-to-cell and systemic movement, as well as homologous CP interactions and particle formation, but is not required for virus replication. Lack of production of the 28kDa CP by either internal initiation or proteolytic cleavage limits systemic infection.

13 HETEROLOGOUS EXPRESSION AND PRODUCTION OF A TOBACCO N (DISEASE RESISTANCE) PROTEIN AND ITS DOMAINS

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Research has been developing for an extensive period of time to develop the biological explanations for the interactions between plants and plant pathogens, but much is to be learned about the molecular nature of these conflicts. Tobacco mosaic virus (TMV) has resulted in major crop losses in the Southern United States and the Netherlands where tobacco is a cash crop (Sholthof, 2004). Avirulent interaction with a tobacco resistance protein enables the elicitation of a hypersensitive response, culminating in cascading events that prevents further infection and initializes local cell death (Dangle, 2001). In order to study these processes, sufficient supply of the tobacco N protein must be present. Previous attempts of production have not yielded usable protein in a high enough concentration. This study successfully used yeast as a heterologous expression system to produce the tobacco resistance gene (N) in a

soluble form allowing further biochemical and molecular biology studies of the specific interactions between TMV and the N gene.

14 A COMPARISON OF SCREENING METHODS FOR *CYMBIDIUM MOSAIC VIRUS* IN ORCHIDS

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Orchid producers worldwide are being pressured by public awareness to test for the presence of *Cymbidium mosaic virus* (CymMV) in their stock. CymMV is the most prevalent disease in cultivated orchids and is characterized by mottling, necrotic lesions, and reduced plant vigor. In this study, screening techniques are compared for efficiency, ease of use, and economic advantage. Several species of bioassay plants (*Cassia occidentalis, Cassia obtusifolia, Datura stramonium, Chenopodium amaranticolor,* and *Tetragonia tetragonioides*) are compared. The tissue samples are also subjected to testing by ImmunoStrips (Agdia, Inc., Elkhart, IN 46514). The presence of CymMV is confirmed by RT-PCR in both the bioassay plant lesions and the plants tested by ImmunoStrip. Both ImmunoStrips and RT-PCR offer faster results than bioassay. However, ImmunoStrips can be utilized by untrained personnel without special facilities, whereas RT-PCR requires skilled personnel with access to laboratory facilities and instrumentation.

15 RTE3: A NOVEL PROTEIN THAT IS A MEMBER OF A NOVEL PROTEIN COMPLEX IN ARABIDOPSIS

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Nuclear pore complexes play a vital role in nuclear-cytoplasmic shuttling, an essential process for proper growth and development in eukaryotes. However, not much is known about these complexes in plants, compared to other organisms. *Arabidopsis RTE3* (*REVERSION-TO-ETHYLENE SENSITIVITY3*) was originally identified in a genetic screen for ethylene-response

mutants and subsequent analyses have suggested that *RTE3* may play an indirect role in ethylene signaling. RTE3 encodes a novel protein and is hypothesized to be a member of the *Arabidopsis* TREX-2 mRNA export complex based on encoding a Sac3 domain (found in the yeast TREX-2 complex) and on the ability to physically interact with members of the TREX-2 complex (Lu et al., 2010). The *rte3* mutants, however, do not show an mRNA export defect. Therefore, it is possible that RTE3 may have a non-essential function in mRNA export and/or an additional function beyond the TREX-2 complex. To gain insight into the molecular function of RTE3, we are exploring protein-protein interactions further. Our bimolecular fluorescence complementation (BiFC) as well as yeast 2-hybrid data suggest that RTE3 physically interacts with the following proteins: EER5 (ENHANCED-ETHYLENE-RESPONSE5), which is in the Arabidopsis TREX-2 mRNA export complex, the C-terminus (residues 516-1294; CEND) of ETHYLENE-INSENSITIVE2 (EIN2), a central player in the ethylene signaling cascade, and CSN3, a subunit of the COP9 signalosome. Genetic analyses reveal an interaction between RTE3 and EER5, and possibly between RTE3 and EIN2. Since EIN2 and CSN3 are not known to be involved in nuclear export, it is possible that RTE3 is part of a conserved protein complex that may have an additional function beyond mRNA export. Further investigation of the protein interactions with RTE3 will not only give insight into the molecular function of RTE3 and the other interacting proteins, but will also give insight into the *Arabidopsis* TREX-2 complex and how it differs from both the mammalian and yeast TREX-2 complexes. In addition, it may give insight into a potential role of RTE3 in ethylene signaling.

16 HARNESSING EUKARYOTIC PROMOTERS FROM VIRUSES INFECTING MARINE INVERTEBRATES

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Promoters are genomic DNA sequences located upstream of an expressed gene (messenger RNA or mRNA) and known to facilitate initiation of transcription. Viruses infecting eukaryotes are generally explored as sources of stronger, constitutive, organ or tissue non-specific promoters. Promoter sequences from viruses such as simian virus 40, cytomegalovirus, or cauliflower mosaic virus are commonly used in cross-kingdom gene expression studies of eukaryotic organisms. However, availability and patent limitations often restrict their use in gene expression studies. We have isolated and characterized three promoters (P2, P11 and P61) from a parvovirus, *Penaeus stylirostris* densovirus (PstDNV) that infects marine shrimp (*Penaeus* sp). These promoters are functional in bacteria, insect (sf9), fish, and shrimp cells. Sequence analysis revealed motifs such as the TATA- box, downstream promoter element (DPE), GC-and A-rich regions, inverted repeat, ASL-box, and a conserved guanosine (G) at +24 in these promoters. We constructed a series of deletion constructs carrying different motifs and

delineated the role of these motifs in modulating transcriptional activities *in vitro.* The cross kingdom functionalities of these promoters are attractive to determining if these promoters can drive gene expression in other eukaryotic hosts (fungi, plants and mammals) and pathogens.

17 *AGROBACTERIUM*-MEDIATED TRANSFORMATION OF *FUSARIUM OXYSPORUM* f.sp. *GLADIOLI* TO STUDY PATHOGENESIS IN *GLADIOLUS*

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Fusarium rot caused by *Fusarium oxysporum* f.sp. *gladioli* (FOG) is one of the most serious diseases of gladiolus, both in the field and in storage. We have transformed the pathogen with *Agrobacterium tumefaciens* binary vectors containing the hygromycin phosphotransferase (*hph*) gene and fluorescence reporter genes of EGFP (green), EYFP (yellow) or ECFP (cyan) in the AGL1 strain of *A. tumefaciens*. Hygromycin (100µg/ml) resistant colonies were observed only when acetosyringone was added to the co-cultivation medium. Transformed colonies are more clearly visible when co-cultivated on cellophane membrane than on Hybond -N+ membrane. Transformed isolates were stably maintained through four serial passages in medium containing hygromycin and expressed green, yellow or cyan fluorescence for the respective gene inserts. PCR with *hph*-specific primers and Southern blotting with *hph*-specific probe were positive from hygromycin resistant isolates but not from the untransformed isolates. The cyan fluorescence of the ECFP-transformed isolate was clearly distinguishable from the green autofluorescence of gladiolus roots, signifying potential of those isolates for further histopathological investigations. Transformed isolates will also be useful for identifying pathogenicity related genes, in the evaluation of transgenic resistance in gladiolus and in studies of host-pathogen interactions.

18 HOW DO PLANTS MAKE CYTOTOXINS WITHOUT CRIPPLING THEMSELVES? STEROIDAL GLYCOALKALOID BIOSYNTHESIS IN WILD POTATO

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Steroidal glycoalkaloids (SGAs) are secondary metabolites of potato and tomato that function in the plant as chemical defenses against Colorado potato beetle and other herbivores. These compounds are found in potato tubers and unripe tomato fruit where they contribute the familiar bitterness associated with the organoleptic properties of these foods. With the overall goal of enhancing the natural pesticide defense of cultivated potato, we are investigating how wild potato (*Solanum chacoense*) makes and uses SGAs. Our objective is to characterize the enzymes that SGA biosynthesis shares with the formation of other metabolites such as phytosterols and sesqui- and triterpenes. As well, we want to identify and characterize the unknown specialized enzymes that catalyze the conversion of cholesterol into SGAs.

S. chacoense (accession 8310-1) makes seven different SGAs. We have monitored the accumulation of these SGAs in all tissues during a time course of development and overlaid a transcript profile of selected genes encoding both the primary and specialized enzymes. Our results indicate that SGA biosynthesis is localized to rapidly growing tissues such as the shoot, floral and stolon meristems.

Squalene synthase (SQS) is a well-characterized enzyme found in all eukaryotes that condenses two molecules of farnesyl diphosphate head-to-head producing the C_{30} hydrocarbon squalene. We targeted plant SQS for further study because (1) the membrane-localized enzyme uses a soluble substrate to produce a hydrophobic product, (2) the enzyme is located at one branch point and just prior to another branch point in the shared pathway of sesquiterpene/phytosterol/triterpene biosynthesis, and (3) whereas a single gene codes for the enzyme in animals, most plants have two genes. Four SQS genes are present in the recently published genome sequence of diploid potato and we have isolated cDNAs for three genes from *S. chacoense*. All three genes have an intron in the 3' UTR that is proximal to the stop codon. We propose that this atypical feature works in mRNA proofreading to ensure that SQS activity is targeted to the correct membrane in the cell.

We have developed high throughput methods to suppress targeted gene expression and assay the phenotype *in planta*. We use a ligation-independent binary cloning vector and *Agrobacterium rhizogenes*-mediated transformation to generate phenotypes in hairy root cultures of wild potato.

19 COMPARATIVE GENOMICS ANALYSIS TO IDENTIFY LETHAL GENES IN ROOT KNOT NEMATODE (*MELOIDOGYNE SPP*) FROM *C. ELEGANS*

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Root Knot nematode (RKN; *Meloidogyne spp.*) is one of the most devastating obligate parasites that infect the roots of thousands of plant species. In the United States, the cost of the losses that these parasites cause annually is estimated to be about $8 billion. The RKN genome sequence is not well characterized or fully sequenced. We sought to identify genes that are lethal at any stage of RKN development. These genes could act as targets for RNAi constructs or gene knockout experiments. To that end we compared RKN sequences to the well-characterized and fully sequenced genome of the free-living nematode *C. elegans* (*Caenorhabditis elegans*). The primary database for *C. elegans* (www.wormbase.org) contains a huge repository of RNAi and mutation experiments on *C. elegans* genes. We conducted a comparative genomics analysis, comparing RKN genes of six species: *Meloidogyne arenaria*, *Meloidogyne chitwoodi*, *Meloidogyne hapla*, *Meloidogyne incognita*, *Meloidogyne javanica*, and *Meloidogyne Paranaensis* to *C. elegans* via wormbase. The RKN sequences were obtained from nematode.net. Our analysis yielded many candidate lethal genes between *Meloidogyne spp* and C.elegans. Some of these genes have already been tested experimentally and found to be indeed lethal to RKN. We also created a relational web based database that links the RKN genes and proteins to their *C. elegans* homologs. The database provides search capabilities for scientists in the field. The database was created using SQL-Server 2008. The web based user interface was created using ASP.NET.

20 ANALYSIS OF BLUEBERRY 454 EST SEQUENCING DATA FROM DIFFERENT STAGES OF FRUIT DEVELOPMENT AND FLOWER BUDS AT FOUR DIFFERENT STAGES OF COLD ACCLIMATION

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Blueberry (*Vaccinium corymbosum*) is a major berry crop in the United States, and one that has great nutritional and economical value. Next generation sequencing methodologies, such as 454, have been demonstrated to be successful and efficient in producing a snap-shot of transcriptional activities during an organism's developmental stage(s) or its response to biotic or abiotic stresses. We have applied a high-throughput pyrosequencing technology (454 EST sequencing) for transcriptome profiling of blueberry during different stages of fruit development to gain an understanding of the genes that are up or down regulated during this process. We have also sequenced flower buds at four different stages of cold acclimation to gain a better understanding of the genes and biochemical pathways that are up- or down-regulated during cold

acclimation, since extreme low temperatures are known to reduce crop yield and cause major losses to US farmers. We have also sequenced a leaf sample to compare its transcriptome profile with that of bud and fruit samples. Over 500,000 sequences were generated and assembled into approximately 15,000 contigs. The contigs and singlet sequences from our analysis were annotated and functionally mapped to Gene Ontology (GO) terms. A database was developed to house these sequences and their annotations. A web based interface was also developed to allow collaborators to search\browse the data and aid in the analysis and interpretation of the data. The availability of these sequences will allow for future advances, and will aid in the blueberry genome sequencing effort that is underway.

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Exploration of the *accelerated cell death 6* mutant in a genetic dissection of defense signaling networks in Arabidopsis

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ABSTRACT

The defense signaling networks have become increasingly complex with the discovery of a growing number of defense regulatory genes and the crosstalks among defense signaling pathways. One hub of the defense networks is centered on salicylic acid (SA), which plays a critical role in signaling disease resistance in plants. Here we exploited the unique defense-sensitive Arabidopsis mutant *accelerated cell death 6* (*acd6-1*) to genetically dissect functional relationships among key components in the SA hub. We show that while *enhanced disease susceptibility* (*eds*) *1-2* and *phytoalexin deficient* (*pad*) *4-1* suppressed *acd6-1*-conferred small size, cell death, and defense phenotypes, a combination of these two mutations did not incur additive suppression, suggesting that *EDS1* and *PAD4* act in the same signaling pathway. To further evaluate genetic interactions among SA regulators, we constructed ten pair-wise crosses in the *acd6-1* background among mutants defective in *SA INDUCTION-DEFICIENT 2* for SA biosynthesis, *AGD2-LIKE DEFENSE 1*, *EDS5*, and *PAD4* for SA accumulation, and *NONEXPRESSOR OF PR GENES 1* for SA signaling. Systematic analysis of the triple mutants based on their suppression of *acd6-1*-conferred phenotypes revealed complex genetic interactions among genes in the SA hub. Our data also suggest that *acd6-1* is a powerful tool that together with additional complementary approaches related to Molecular Genetics, Biochemistry, and global gene expression profiling should allow us to systematically interrogate the complex defense signaling networks and to better understand the mechanisms of plant disease resistance.