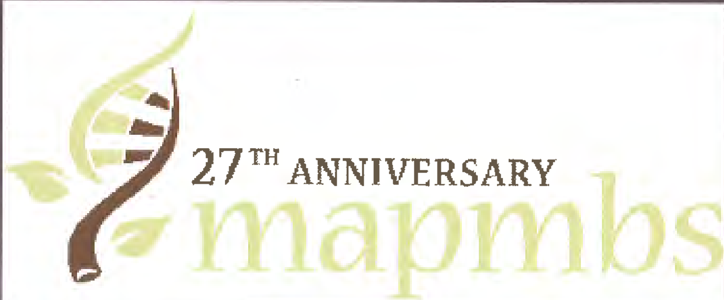
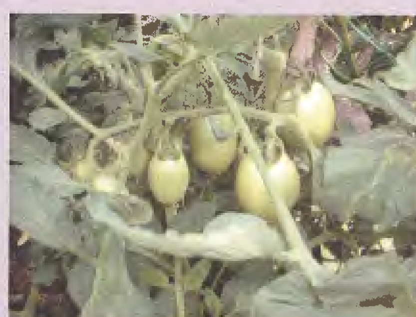


27th Annual Mid-Atlantic Plant Molecular Biology Society Meeting

12th & 13th August, 2010

National Wildlife Visitor Center,
Patuxent Research Refuge
Laurel, MD



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WELCOME

WELCOME

On behalf of the Organizing Committees we welcome you to the twenty-seventh annual meeting of the Mid-Atlantic Plant Molecular Biology Society in 2010. 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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MAPMBS 2010 PROGRAM

THURSDAY, AUGUST 12, 2010

9:00 Registration and poster set-up

9:20 Welcome (Ben Matthews and Leslie Wanner)

SESSION I **CLIMATE CHANGE and AGRICULTURE**

Introduction & moderator, **Bret Cooper**, USDA-ARS, Beltsville MD

9:25 **Molly Brown**, Biospheric Sciences Branch, NASA Goddard Space Flight Center, Greenbelt, MD
The impact of climate change on agriculture and food security

10:00 **Carole Bassett**, USDA- ARS, Appalachian Fruit Research Station, Kearneysville, WV
The CBF gene family in apple (Malus x domestica Borkh.)

10:25 **Thomas Malvar**, Monsanto Co., Mystic, CT
Gene discovery for drought stress tolerance in maize

11:00 **COFFEE BREAK:** Poster Session and Exhibitors

SESSION II **UNDERSTANDING and MODIFYING PLANT GENOMES**

Moderator: **Jim Saunders,** Towson University, Baltimore MD

11:20 **Bailin Li**, DuPont Agricultural Biotechnology, Wilmington, DE
Association mapping, QTL mapping and positional cloning in maize: genes discovery for crop improvement

11:55 **Steve Mount,** Dept. Cell Biology and Molecular Genetics, University of Maryland, College Park, College Park, MD
***The open immature flower phenotype in hybrids between the Landsberg erecta and Columbia strains of* Arabidopsis thaliana**

12:20 MAPMBS business meeting and planning for next year

12:35 **Lunch, posters, exhibits**

SESSION II (cont.) **UNDERSTANDING and MODIFYING PLANT GENOMES**

moderator, Jim Saunders, Towson University, Baltimore MD

1:50 **Songqing Ye**, Department of Horticultural Science and the Microbial and Plant Genomics Institute, University of Minnesota
ATP synthase β is potentially modified by auxin during strawberry fruit development

2:15 **Scott Warnke**, USDA-ARS, Floral and Nursery Plants Research Unit, Beltsville MD
Miniature inverted repeat transposable elements (MITEs) in Agrostis: Important evolutionary force or junk DNA

2:50 **COFFEE BREAK:** Poster Session and Exhibitors

3:20 **Introduction of Keynote Speaker,** John Hammond, USDA-ARS, Floral and Nursery Plants Research Unit, Beltsville MD

3:25 **KEYNOTE ADDRESS: Jeffrey Bennetzen**
Department of Genetics, University of Georgia, Athens, GA

Contributions of selfish and useful transposable elements to the evolution of genome structure and function in plants

4:30 Close of Day (Building closes)

http://bioinformatics.towson.edu/mapmbs/default.aspx

FRIDAY, AUGUST 13

9:00 **REGISTRATION, POSTER SESSION and EXHIBITORS**

SESSION III **DEFENSE AGAINST PESTS and PATHOGENS**
moderator, Reid Frederick, USDA-ARS Frederick, MD

9:30 **Stephen Streatfield**, Fraunhofer USA Center for Molecular Biotechnology,Newark, DE
Plant based vaccine technology suited for rapid responses: influenza

10:05 **Gary W. Felton**, Department of Entomology, Penn State University, University Park, PA
Manipulation of plant defenses by insect salivary effectors

10:40 **COFFEE BREAK: POSTER SESSION and EXHIBITORS**

11:10 **Arianne Tremblay**, Soybean Genomics & Improvement Laboratory, USDA-ARS, Beltsville, MD
Transcriptome analysis of a susceptible genotype of* Glycine max *during a time-course of infection with* Phakopsora pachyrhizi *using next generation sequencing

11:25 **Anna Maria Vaira**, CNR, Istituto di Virologia Vegetale, Torino, Italy and USDA-ARS, US National Arboretum, Floral & Nursery Plants Research Unit, Beltsville, MD
Chloroplast targeting and virus systemic movement: associated features promoted by different viral proteins in two distinct flexiviruses

12:10 **LUNCH: POSTER SESSION and EXHIBITORS**

SESSION III, cont. **DEFENSE AGAINST PESTS and PATHOGENS**
moderator, David Puthoff, Frostburg State Univ. Frostburg, MD

1:20 **Mike Timko,** Department of Biology, University of Virginia, Charlottesville, VA
Race-specific resistance to* Striga gesnerioides *in cowpea: A case of effector triggered immunity?

1:55 **Shiv Kale**, Virginia Bioinformatics institute, Virginia Tech, Blacksburg, VA
Mechanism of oomycete and fungal effector protein translocation into plant host cells

2:30 **Heba Ibrahim**, USDA-ARS, Soybean Genomics & Improvement Laboratory, Beltsville, MD and Genetics Department, Faculty of Agriculture, Cairo University, Giza, Egypt
Developing Soybean Plants with Resistance to Root-Knot Nematode Using Biotechnology

2:55 **Close of day: Thanks for your participation! Please take down your poster**

Poster #	Abstract Page	Author(s); affiliation: *TITLE*
01	23	**C. Srinivasan, Zongrang Liu** and **Ralph Scorza** USDA- ARS, Appalachian Fruit Research Station, Kearneysville, WV ***ECTOPIC EXPRESSION OF CLASS 1 KNOX GENES INDUCE ADVENTITOUS SHOOT REGENERATION AND ALTER GROWTH AND DEVELOPMENT OF TOBACCO (Nicotiana tabacum L) AND EUROPEAN PLUM (Prunus domestica L)***
02	23	**Natalia Kovalskaya[1,2], Yan Zhao[1]** and **Rosemarie W. Hammond[1]** [1]USDA-ARS Molecular Plant Pathology Laboratory, Beltsville, MD 20705 [2]Institute of Ecological Soil Science of MV Lomonosov Moscow State University, Moscow 119899, Russia ***ANTIMICROBIAL ACTIVITY OF SNAKIN-1 AND DEFENSIN-1 AS A HYBRID PROTEIN IN IN VITRO AND IN VIVO EXPERIMENTS AGAINST PLANT PATHOGENS***
03	24	**Ruiqiang Chen[1], Caren Chang[1], Mark L. Tucker[2] and Bret Cooper[2] *** [1]Dept. of Cell Biology and Molecular Genetics, Univ. of Maryland, College Park, MD 20742; [2]USDA-ARS Soybean Genomics and Improvement Laboratory,Beltsville, MD 20705 ***PROTEOMIC ANALYSIS OF ETHYLENE HORMONE SIGNALING IN Arabidopsis***?
04	25	**Angela Natilla** and **Rosemarie W. Hammond[1]** USDA, ARS, Molecular Plant Pathology Laboratory, Beltsville, MD 20705 ***CYSTEINE MUTANTS OF MAIZE RAYADO FINO VIRUS-LIKE PARTICLES AS A PLATFORM FOR MULTIVALENT DISPLAY***
05	26	**Tyler Lancaster** and **David Puthoff** Frostburg State University, Department of Biology, 101 Braddock Rd, Frostburg, MD 21532 ***METHODS FOR INCREASING LEVELS OF TERPENOID ACTIVE INGREDIENT(S) IN BLACK COHOSH (ACTAEA RACEMOSA) AND ELUCIDATION OF THEIR ROLE IN PLANT DEFENSE***
06	26	**Elizabeth S. Johnson[1,2],, Dapeng Zhang[1]*, Winson July Martinez[3], Eduardo Somarriba[4], Wilbert Philips-Mora[3], Carlos Astorga[3], Sue Mischke[1] and Lyndel W. Meinhardt[1]** [1]USDA-ARS- SPCL, 10300 Baltimore Ave, Bldg 001, Rm 223, Beltsville, MD 20705; [2]current address: CABI Caribbean and Latin America, Gordon Street, Curepe, Trinidad and Tobago, W.I [3]Laboratorio de Biotecnología, CATIE 7170, Turrialba, CR [4]Agroforestry & Watershed Management, CATIE 7170, Turrialba, CR ***GENETIC DIVERSITY AND SPATIAL STRUCTURE IN CACAO GERMPLASM FROM BOLIVIA***
07	27	**Ji Kun[1, 2], Dapeng Zhang[1], Stephen Pinney[1], Sue Mischke[1], Lambert Mortilal[3], and Lyndel W. Meinhardt[1]** [1]USDA-ARS, SPCL, 10300 Baltimore Avenue, Beltsville, MD 20705, USA. [2]School 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 ***IDENTIFICATION OF TRADITIONAL CACAO VARIETIES USING MICROSATELLITE AND SINGLE NUCLEI***

08 28

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 Fauquet[2], John Hammond[1]
[1]USDA-ARS, FNPRU, Beltsville, MD 20705; [2]Danforth Plant Science Center, St. Louis, MO 63132; [3]University of Utah, Salt Lake City, Utah 84112; [4]Washington University, St. Louis, MO 63110; [5]Cornell University, Ithaca, NY, 14853; [6]Oklahoma State University, Stillwater, OK 74078

ISOLATION OF TOTAL NUCLEIC ACID FOR MICROARRAY-BASED DETECTION OF VIRUSES IN PLANTS

09 28

Bruno V. Oliveira[1], Goncalo A. Pereira[1], Bryan A. Bailey[2] , Lyndel W. Meinhardt[2]
[1]Laboratorio de Genomica e Expressao, Universidade Estadual de Campinas, Campinas, Brasil; [2]USDA-ARS, SPCL, 10300 Baltimore Ave, Bldg 001, Rm 223, Beltsville, MD 20705

EXPRESSION ANALYSIS OF AN ALCOHOL OXIDASE GENE FROM THE FUNGUS* MONILIOPHTHORA RORERI*, THE CAUSAL AGENT OF CACAO FROSTY POD ROT

10 29

Christine L. Stone and Reid D. Frederick
USDA-ARS, Foreign Disease-Weed Science Research Unit, Fort Detrick, MD 21702

SOYBEAN RUST GENE EXPRESSION DURING APPRESSORIUM FORMATION

11 30

Parsa Hosseini[1], Arianne Tremblay[2], Nadim W. Alkharouf[1], Benjamin F. Matthews[2]
[1]Department of Computer and Information Sciences, Towson University, Towson, MD 21252; [2]USDA-ARS, Soybean Genomics & Improvement Laboratory, Beltsville, MD 20705

***ANALYSIS OF* GLYCINE MAX *MRNA-SEQ DATA UPON A TIME-COURSE INFECTION WITH* PHAKOPSORA PACHYRHIZI**

12 30

Robert Farrell[1,3], Jacob Moore[1], Ryan Jenkins[1], and Carole Bassett[2]
[1]Department of Biology, Penn State University, York, PA
[2]Appalchian Fruit Research Station, Kearneysville, WV

IDENTIFICATION OF DROUGHT RESPONSIVE GENES IN APPLE BY MODIFIED GENE SUBTRACTION

13 31

Courtney A Hollender[1], Janet Slovin[2], Zhongchi Liu[1,2]
[1]Dept. of Cell Biology and Molecular Genetics, University of Maryland, College Park; [2]USDA-ARS, Genetic Improvement of Fruit and Vegetables Laboratory, Beltsville, MD

STRAWBERRY: A NEW MODEL FOR STUDYING FLOWER AND FRUIT DEVELOPMENT

THE IMPACT OF CLIMATE CHANGE ON AGRICULTURE AND FOOD SECURITY

Molly E. Brown, C.C. Funk, G. Eilerts

Biospheric Sciences Branch, Code 614.4, Goddard Space Flight Center Greenbelt, MD 20171

molly.brown@nasa.gov

By 2050, the human population is likely to peak at nearly 10 billion and the impacts of climate change will be widespread. This paper examines current trends in food production in the face of these trends. The analysis is based on the idea of per capita agricultural capacity, which is measured as a ratio of population and large-scale agricultural factors (harvested area, seed use, and fertilizer use). This framework allows for the understanding of concurrent trends in population growth and agriculture technology on local food production. Along with an analysis of climate and food security trends in Africa, the paper explores global per capita agricultural capacity trends through the use of statistics and analysis. The analysis shows that stagnating yields due to climate change and limited agricultural development will lead to substantial declines in per capita cereal production in parts of Africa, Asia, and Central and Southern America. Food prices will likely increase as the current highly productive agricultural regions in the US, Europe and Asia are unable to keep up with demand. Global per capita cereal production will potentially decline by 14 percent between 2008 and 2030. Unless population growth slows or the adoption of improved seeds and fertilizer accelerates, the competition for food will grow. Low income developing countries will have to compete directly with high income population centers in other parts of the world, causing further food security problems due to food being priced out of reach of many of the poor. This emerging crisis also offers an opportunity for growth: in addition to adding to global agricultural capacity, increasing local agricultural productivity in currently food insecure zones is also an effective way for the very poor in developing countries to gain access to higher incomes and more secure sources of food.

THE CBF GENE FAMILY IN APPLE (MALUS x DOMESTICA BORKH.)

Carole L. Bassett[1], Michael E. Wisniewski[1], Mickael Malnoy[2], Riccardo Velasco[2] and John L. Norelli[1]

[1] USDA, ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430
[2] FEM-IASMA Research Centre, Via E. Mach 1, 38010 San Michele all'Adige (TN) Italy

Carole.Bassett@ars.usda.gov

Many vascular plants have evolved mechanisms for protecting themselves from freeze damage. One of the key pathways controlling higher plant responses to low temperature involves a family of genes which belong to the AP2 domain class of transcription factors. The promoters of many genes involved in abiotic stress responses contain specific elements known as cold repeat (C-repeat)/dehydration response elements (DRE) to which these transcription factors (called CBF/DREB for C-repeat or dehydration responsive binding factors) bind to regulate transcription. In *Arabidopsis thaliana* there are four members of this family which regulate cold (CBF1, 2 and 3) and dehydration (CBF4) stress responses. We have identified seven genes from 'Golden Delicious' apple (Whole Genome Sequence project, FEM-IASMA, Italy) encoding AP2 genes homologous to the Arabidopsis CBF genes. Five of these genes appear to encode full length sequences corresponding primarily to the Arabidopsis CBF2 and 4 genes. Two of the apple genes encode polypeptides truncated at the N-terminus due to alterations in their sequences which eliminate the 5'-most ATG. We present an analysis of this family and comparison to the CBF/DREB1 genes of Arabidopsis.

GENE DISCOVERY FOR DROUGHT STRESS TOLERANCE IN MAIZE

Thomas Malvar

Monsanto Co., 62 Maritime Dr., Mystic, CT 06355

New traits in the areas of yield improvement and abiotic stress control will represent a step-change in crop yield performance. Among these, the efficient use of water in agricultural production is one of the greatest challenges, with agriculture currently being responsible for ~70% of freshwater withdrawal. As such, providing yield improvement through tolerance to water deficits that occur routinely in the Central Corn Belt and frequently in western states is critical in the coming decade. The benefits of improving water use efficiency, in addition to higher yield, are expected to include reduced water consumption and environmental sustainability. The ability to test large numbers of genes in both controlled environment and field settings has identified those that impact drought tolerance in corn. This presentation will provide an overview of our effort to develop the next generation biotech products for yield and stress.

ASSOCIATION MAPPING, QTL MAPPING AND POSITIONAL CLONING IN MAIZE: GENES DISCOVERY FOR CROP IMPROVEMENT

Bailin Li

DuPont Agricultural Biotechnology, Wilmington, Delaware

Natural variation is at the core of plant breeding. Genetic or linkage mapping is the traditional method for identifying loci/genes responsible for variation in complex traits. More recently, association mapping or linkage disequilibrium (LD) mapping, which establishes marker-trait association in populations of unrelated individuals, is becoming an alternative approach for gene discovery. We have developed tools and methods for positional cloning as well as genome-wide association analysis in maize, thus enabling the molecular dissection of traits of agronomic interest. An integrated genetic/physical/gene/diversity map has been constructed, with very high genetic marker density, good BAC contig coverage of maize genome, over 21,000 EST anchored on the physical map, as well as 10,000 loci re-sequenced in 600 inbred lines. We have successfully identified loci associated with variations of various traits through whole genome scan association mapping. In some cases, the association has been validated in bi-parental segregating populations, and candidate genes identified. We have also cloned well over a dozen maize mutant genes/QTLs by map-based cloning. The high resolution mapping and cloning of QTL make it possible to develop tightly linked markers for marker-assisted selection, germplasm characterization, screening for new functional alleles, as well as better understanding of the genetic architecture of agronomic traits. I'll present examples of gene isolation by association mapping and map-based cloning in maize.

THE OPEN IMMATURE FLOWER PHENOTYPE IN HYBRIDS BETWEEN THE LANDSBERG ERECTA AND COLUMBIA STRAINS OF ARABIDOPSIS THALIANA

***Stephen M. Mount*[1]**, *Amishav Bresler[1,2], Kevin P. Cilano[3], Dionne Rebello[1] and Xiao-Ning Zhang[1,3]*

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3. Department of Biology, St. Bonaventure University, St Bonaventure, NY 14778

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Mutations in the splicing factor gene *SR45* affect flowering time, root growth and flower morphology, but have only mild effects on fertility. We identified *OPEN IMMATURE FLOWER* (*OIF*) as a natural variant in the Landsberg erecta strain that produces a premature opening of flowers in *sr45-1*; *oif-Ler* double mutants and greatly reduces fertility. Two additional alleles of *OIF*, *oif-1* and *oif-2*, were isolated in the Columbia background as enhancers of *sr45-1* following EMS mutagenesis. These mutations fail to complement *oif-Ler*, which suggests that the Landsberg erecta strain carries a loss-of-function variant at the *OIF* locus.
Scanning electron microscopy indicates that the oif phenotype follows early defects in anther, sepal and carpel development. Compared to the wild type, *sr45-1* mutant flowers have exaggerated inward curving sepals, narrower pollen sacs, produce fewer pollen grains and have delayed fusion of the gynoecia around stage 10. *sr45-1; oif* mutant flowers have even smaller pollen sacs, much less pollen grain production and a significantly abnormal fusion of gynoecia. Premature flower opening appears to result from a combination of the over-curvature of the sepal and the accelerated longitudinal growth of the abnormal carpel, while reduced fertility is likely due to defects in both anther and carpel development.
Surprisingly, we have observed that some F2 from a cross between Landsberg erecta and Columbia show the oif phenotype in their earliest flowers, even though they are wild-type for *SR45*. This observation has now been confirmed by identification of the oif phenotype in some recombinant inbred lines derived from these parent strains (the Lister and Dean Columbia x Landsberg collection), suggesting that Columbia carries a natural variant that enhances oif-Ler. We are currently mapping both *OIF* and the putative enhancer of oif.

ATP SYNTHASE Β IS POTENTIALLY MODIFIED BY AUXIN DURING STRAWBERRY FRUIT DEVELOPMENT

Songqing Ye[1,2,3], Seijin Park[1], Zhongchi Liu[2], Janet P. Slovin[3] and Jerry D. Cohen[1]

[1]Department of Horticultural Science and the Microbial and Plant Genomics Institute, University of Minnesota
[2]Department of Cell Biology and Molecular Genetics, University of Maryland
[3]USDA-ARS Genetic Improvement of Fruit and Vegetables Laboratory, Beltsville, MD

Auxin has diverse roles that regulate many aspects of plant growth and development. The auxin indole-3-acetic acid (IAA) regulates fruit development in strawberry where auxin from the achenes exerts growth control over the receptacle. The majority of auxin is present in conjugated forms in plants and auxin conjugates are thought to serve as reservoirs of inactive IAA readily convertible to the active form, as a form for long distance transport of IAA, or as intermediates in IAA turnover, such as IAA-Asp in *Arabidopsis*. Several auxin conjugates, including IAA linked to amino acids, sugars, and peptides, have been identified. Proteins that are covalently linked to IAA have been studied in bean and strawberry. A strawberry fruit protein extract was screened using a specific IAA-Gly-BSA antiserum. IAA modified proteins with strong immune-reaction were identified by searching the protein database following LC-MS/MS and tentatively identified as an ATP synthase β. Two genes for ATP synthase subunit β have been found in strawberry by alignment with the three Arabidopsis genes. Those genes have now been cloned and expressed in *E. coli*. Enzymatic assays of these proteins are currently being performed with the goal of determining if the indole-acyl modification has a functional role. Comparisons will be made of the activity of cloned, unmodified enzymes to the activity of proteins obtained using ATP synthase β antiserum and IAA-Gly-BSA antiserum.

]This work is supported by NSF grant (IOS-0820940) awarded to JD Cohen and JP Slovin.

MINIATURE INVERTED REPEAT TRANSPOSABLE ELEMENTS (MITES) IN AGROSTIS: IMPORTANT EVOLUTIONARY FORCE OR JUNK DNA

Scott E. Warnke and Keenan L. Amundsen

USDA-ARS, Floral and Nursery Plants Research Unit, Beltsville, MD

Scott.Warnke@ars.usda.gov

Creeping bentgrass (*Agrostis stolonifera* L.) is the premier turfgrass species used on golf course putting greens, fairways, and tees because of its hardiness, growth and aesthetic characteristics and ability to tolerate low mowing. The genus *Agrostis* is a polyploidy series with genome numbers ranging from 2X to 10X. Creeping bentgrass is a highly outcrossing allotetraploid (2n=4x=28) with the genome designation A2A2A3A3. The diploid species possessing the A2 and A3 subgenomes of creeping bentgrass are currently unknown. One of the difficulties in determining the diploid progenitors of the cultivated bentgrasses is the lack of accurate ploidy determinations for many of the Agrostis accessions maintained in the National Plant Germplasm System (NPGS). The objective of this research was to assess the ploidy level and the amount of genetic diversity present in a subset of the NPGS *Agrostis* germplasm. In the present study, 75 *Agrostis* accessions representing 15 distinct species along with two *Apera* and four *Polypogon* accessions were screened using flow cytometry and 1,309 Miniature Inverted repeat Transposable Element (MITE) display DNA markers. Cluster analysis clearly separated the common turf-type *Agrostis* species into distinct groups. The inclusion of previously understudied species within these groups offers insights into the genomic origins of creeping bentgrass that may be useful in future breeding efforts. In addition, the data suggest a narrowing of the genetic diversity within cultivated creeping bentgrasses.

KEYNOTE ADDRESS

CONTRIBUTIONS OF SELFISH AND USEFUL TRANSPOSABLE ELEMENTS TO THE EVOLUTION OF GENOME STRUCTURE AND FUNCTION IN PLANTS

Jeff Bennetzen

Department of Genetics, University of Georgia, Athens, GA

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Transposable elements (TEs) are nearly ubiquitous in nature, and they constitute the majority of the nuclear genomes of most flowering plants. Even in those plants with tiny genomes, like those of Arabidopsis and rice, TEs are the most dynamic genome component, actively involved in the modification of chromosome structure, gene content, gene regulation and gene function. Many TEs have been domesticated for specific genic or genomic uses, but the majority behave as selfish entities that are competing for survival in a complex and highly competitive environment. This balance between selfish and useful outcomes for TEs requires further investigation, particularly from the standpoint of the possible differing needs of different lineages for differing ratios of continuity versus change in their genetic material.

PLANT-BASED VACCINE TECHNOLOGY SUITED FOR RAPID RESPONSES: INFLUENZA

Stephen J. Streatfield

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The concept of expressing target antigens in plant tissues to manufacture bulk drug substances for subunit vaccines was proposed about two decades ago and has since received considerable attention, with extensive academic and applied industrial research. Most published reports have focused on demonstrating target antigen expression and immunogenicity of plant-produced targets following administration to animals, usually mice. Extensive literature supports expression of correctly folded antigens that can induce humoral and cell-mediated immune responses. In several cases vaccine candidates have advanced into efficacy studies using established animal disease models, such as ferrets for influenza, and rabbits and non-human primates for anthrax. A handful of phase I clinical trials have also been completed with the most advanced candidates, including parenterally and orally administered vaccines. In addition, a few animal vaccine candidates have been assessed in target species trials, and in 2006 the USDA approved a plant-based animal vaccine candidate for Newcastle disease in chickens. Currently, a major emphasis in the field is on attaining sufficiently high levels of extractable antigen expression to be compatible with economic downstream processing and purification. Highly purified targets are required for formulation prior to parenteral administration, whereas cruder processing of plant tissues can suffice for mucosal administration, but both applications require robust and consistent antigen expression. Three general technologies have been applied to express high levels of target antigens in plants: nuclear transformation, plastid transformation, and transient approaches, including inoculation with plant virus-based vectors. Transient technologies have the added advantage of allowing for rapid responses to emerging pandemics and novel bioterror threats. This presentation will focus on the application of a plant virus-based transient expression technology to manufacture influenza antigens for formulation into parenterally administered vaccines. The biochemical, biophysical and immunological characterization of purified antigen targets will be described, and the scale-up of the expression system to produce bulk drug substances under cGMP for clinical trials will be reported.

MANIPULATION OF PLANT DEFENSES BY INSECT SALIVARY EFFECTORS

Gary W. Felton

Department of Entomology, Penn State University, University Park, Pennsylvania, USA

Herbivores leave a "chemical trail" as they feed on plants. The oral secretions left behind on the trail may aid the herbivore in exploiting its host. Using tomato and tobacco as a model systems, we are investigating plant recognition of herbivores using a variety of molecular and pharmacological approaches. Our studies indicate that some insect herbivores my effectively intercept some of these defense signals and thus partially evade plant defenses, whereas other insect herbivores may inadvertently amplify these defense messages. We are using a proteomic approach to identify these secretions and are studying the genes that encode them. Our findings indicate that the dialogues occurring at the plant-insect interface are considerably more complex than previously known.

TRANSCRIPTOME ANALYSIS OF A SUSCEPTIBLE GENOTYPE OF Glycine max DURING A TIME-COURSE OF INFECTION WITH Phakopsora pachyrhizi USING NEXT GENERATION SEQUENCING

Arianne Tremblay[1], Parsa Hosseini[1,2], Nadim W. Alkharouf[2], Shuxian Li[3], Douglas G. Luster[4], Benjamin F. Matthews[1]

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Soybean is in the top five agricultural products in the United States. Soybean rust (SR) is caused by an exotic obligate fungus. We want to analyze the expression pattern of SR and its soybean host genes during the infection. Thus, libraries were constructed from different soybean cells infected by SR at different time-points and sequenced using a Solexa platform. Infection sites were visualized by immunofluorescence and isolated by laser capture microdissection. DNA sequences were aligned to the soybean genome and homology searches were conducted to determine the identity of the genes. From sequences without similarity to soybean genome, contigs were formed and homology searches were conducted. All the time-points give us a limited number of sequences aligning to the soybean genome (3,330 sequences/time-point). However, we found an average of 9,683,207 sequences per time-point without any homology to the soybean genome. Most of these are expected to be SR sequences. Some of the contigs built from these sequences have homology 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 do not share similarity with genes in NCBI database but a manual homology search against different domain databases gives 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. Target pathogen as well as some relevant host genes will be studied to determine if they can be used to control SR in soybean.

CHLOROPLAST TARGETING AND VIRUS SYSTEMIC MOVEMENT: ASSOCIATED FEATURES PROMOTED BY DIFFERENT VIRAL PROTEINS IN TWO DISTINCT FLEXIVIRUSES

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Lolium latent virus (LoLV) and *Alternanthera mosaic virus* (AltMV) belong to the family *Flexiviridae*, and are respectively the type species of the genus *Lolavirus*, and a member of the genus *Potexvirus*. LoLV virions are composed of equal amounts of two coat proteins, the larger being an in-frame N-terminal extension of the smaller; this N-terminal region includes a 42 amino acid sequence encoding a putative chloroplast transit peptide (cTP). Localization studies reveal that this region is required for mesophyll and chloroplast coat protein targeting; the C-terminal region of the coat protein is immuno-detected inside purified chloroplasts following trypsin treatment, suggesting internalization and cleavage of the large protein at the chloroplast. Mutation of the first ATG in an infectious clone leads to expression of the small coat protein only, lacking the cTP region. The mutation of the first ATG does not affect replication or cell-to-cell movement, but completely abolishes systemic infection, that in some cases is naturally recovered by various point mutations which restore a longer coat protein. In AltMV, TGB3 preferentially localizes in mesophyll cells; deletion and site-specific mutagenesis revealed an internal dipeptide signal essential for chloroplast localization. Either deletion of the TGB3 start codon or alteration of the chloroplast localization signal limited cell-to-cell movement of mutated infectious clones to the epidermis, yielding a virus that was unable to move into the mesophyll layer.
In each case chloroplast targeting is associated with virus ability to invade the mesophyll and achieve systemic movement, through different viral proteins and different mechanisms. This targeting may reflect the necessity for these two viruses to reach the preferential site for replication or viral assembly, or to colonize the areas surrounding the vascular system, in order to accomplish long distance movement. Effect on cell metabolism may also take place following these interactions. Host factor interaction studies are in progress to identify host proteins involved in the association with chloroplasts.

RACE-SPECIFIC RESISTANCE TO* STRIGA GESNERIOIDES *IN COWPEA: A CASE OF EFFECTOR TRIGGERED IMMUNITY?

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Cowpea (*Vigna unguiculata* L. Walp.) is a major food and forage legume critical to the nutrition and economy of sub-Saharan Africa. Among the major biotic constraints to cowpea production is the parasitic witchweed, *Striga gesnerioides* (Willd.). Few naturally resistant genotypes exist in wild and domesticated germplasm. Using host differential response trials and molecular genotyping of the parasite genome, we have demonstrated that at least seven distinct races of *Striga* parasitic on cowpea exist in West and Central Africa. Resistant cowpea genotypes exhibit one of two different response mechanisms to *Striga* attack: rapid necrosis at the site of parasite attachment (hypersensitive response - HR), very limited parasite growth and a failure to expand their cotyledons (tubercle arrest - TA). Using a positional cloning strategy, we have isolated a gene, *RSG3-310*, conferring race-specific resistance to *S. gesnerioides* race 3 (SG3) and shown that encodes a CC-NBS-LRR type resistance (R) protein. Silencing of *RSG3-301* results in the loss of the SG3 resistance phenotype, but does not affect other race specific resistances (e.g., SG2 and SG5 resistance) in the multirace resistant cowpea cultivar B301. These findings suggest that a gene-for-gene mechanism characteristic of effector-triggered immunity (ETI) is operating in *Striga*-cowpea interactions. We are using genetics and functional genomic approaches to identify effectors/avirulence factors involved in this process and to unravel the mechanism underlying race-specific resistance to *Striga* parasitism. We hope this knowledge will lead to the design of strategies for improving sustainable resistance in cowpea, as well as other crops suffering under the scourge of the witchweeds. Our progress towards this end and its implications will be discussed.

MECHANISM OF OOMYCETE AND FUNGAL EFFECTOR PROTEIN TRANSLOCATION INTO PLANT HOST CELLS

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Oomycetes and fungal plant pathogens are responsible for billions of dollars worth of damage to agricultural crops annually. One such destructive pathogen is *Phytophthora sojae*, a hemibiotrophic oomycete that causes root rot in soybeans. *P. Sojae* utilizes effector proteins to suppress host cell defense mechanisms, facilitating pathogenesis. Many well-studied effector proteins from oomycetes (PsAvr1b, PiAvr3a, and ATR13) and fungi (AvrL567, Avr2, and AvrM) are known to localize inside host cells and are subsequently recognized by their respective R proteins. Analysis of these oomycete effector proteins reveals a highly conserved N-terminus RXLR-dEER motif. This motif is responsible for the translocation of oomycete effector proteins into host cells via the host's own translocation machinery. We show that the RXLR-motif binds phosphatidyl-3-phosphate (PI-3-P) found in the lipid micro-domains on the surface of the host cell. The binding of PI-3-P mediates entry of these effector proteins into a broad variety of cell types. The detailed mutagenesis of the RXLR-motif shows that the motif retains cell entry activity even with a wide range of substitutions. These functional substitutions have allowed us to generate a new criterion for identifying RXLR motifs. Using this criterion we identify RXLR-like motifs in a variety of well-studied fungal effectors: AvrL567, Avr2, and AvrLm6. We show that these RXLR-like motifs mediate effector cell entry into host cells without any pathogen-encoded machinery by binding PI-3-P. Cell entry can be prevented by either sequestering PI-3-P on the cell surface or by employing 1,3-inositol diphosphate as a competitive inhibitor. By preventing effector entry into host cells we hope to reduce infection by oomycete and fungal plant pathogens on a wide variety of agriculturally important crops.

DEVELOPING SOYBEAN PLANTS WITH RESISTANCE TO ROOT-KNOT NEMATODE USING BIOTECHNOLOGY

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Root-knot nematodes (RKN) are sedentary endoparasites that can infect more than 3000 plant species. RKN cause an estimated $100 billion annually in losses worldwide. We examined gene expression in galls formed by RKN in soybean roots 12 days and 2.5 months after infection to understand the effects of infection of roots by RKN better and to identify possible gene targets for manipulation to develop broad resistance of plants to RKN. Gene expression was monitored using the Affymetrix Soybean GeneChip containing 37,500 *G. max* probe sets. Gene expression data for many enzymes was visualized by integrating the data with biochemical pathways from Kyoto Encyclopedia of Genes and Genomes using PAICE software. A number of different soybean genes were identified that were differentially expressed that provided insights into the interaction between RKN and soybean and into the formation and maintenance of giant cells. According to the expression level of some of those genes and some other genes in many different pathways we found many genes that appear to have essential role in the soybean-RKN interaction. Some of these genes may be candidates for broadening resistance of plants to RKN by over-expression or silencing and will require further analysis and testing.

We also used RNA interference (RNAi) to silence four RKN genes to determine if this approach will provide resistance in soybean to RKN. We identified RKN genes having high similarity with essential soybean cyst nematode (*Heterodera glycines*) and *C. elegans* genes using BLAST. Four RKN genes were chosen according to their expected essential function in the life cycle. The four RKN genes were amplified, cloned, and used to transform soybean to obtain roots expressing RNAi to silence these RKN genes. The transformed roots were recognized by the presence of green fluorescent protein. The transformed roots were challenged with RKN and at different time points we took root samples for staining to monitor nematode infection inside the RNAi transformed roots compared to control roots. After 28 days post infection, we found that two constructs interfered with the life cycle of RKN. The number of galls formed on these roots was significantly reduced by 92% and 94.7%. Also the development of the nematodes inside the transformed roots was retarded and the diameters of the nematodes were noticeably smaller compared to the control. These approaches may be useful to broaden resistance of soybean against RKN.
information is used to complement DNA marker information to map a genetic trait.

http://bioinformatics.towson.edu/mapmbs/default.aspx

01 *ECTOPIC EXPRESSION OF CLASS 1 KNOX GENES INDUCE ADVENTITOUS SHOOT REGENERATION AND ALTER GROWTH AND DEVELOPMENT OF TOBACCO (Nicotiana tabacum L) AND EUROPEAN PLUM (Prunus domestica L)*

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Transgenic plants of tobacco (*Nicotiana tabacum* L) and European plum (*Prunus domestica* L) were produced by transforming with apple class 1 *KNOX* genes (*MdKN1 and MdKN2*) or corn *KNOX1* gene. Transgenic tobacco plants were regenerated in vitro from transformed leaf discs cultured in a culture medium lacking cytokinin. Ectopic expression of *KNOX* genes retarded shoot growth by suppressing elongation of internodes in transgenic tobacco plants. When over-expressed, all three *KNOX1* genes induced malformation and extensive lobbing in tobacco leaves, In situ regeneration of adventitious shoots was observed from leaves and roots of transgenic tobacco plants expressing all three *KNOX* genes. In vitro culture of leaf explants and stem internode sections excised from in vitro grown *MdKN1* expressing tobacco shoots regenerated adventitious shoots on Murashige and Skoog (MS) basal medium in the absence of exogenous cytokinin. Transgenic plum plants that expressed *MdKN2* or corn *KNOX1* gene grew normally but *MdKN1* caused reduction in shoot growth and leaf malformation. A high frequency of adventitious shoot regeneration (95%) was observed in cultures of leaf explants excised from corn *KNOX1* expressing transgenic plum shoots. In contrast to *KNOX1*-expressing tobacco, leaf and internode explants of corn *KNOX1* expressing plum required synthetic cytokinin (thidiazuron) in the culture medium for adventitious shoot regeneration. The induction of high frequency regeneration of adventitious shoots in vitro from leaves and stem internodal sections of plum through the ectopic expression of a *KNOX1* gene is the first such report for a woody perennial fruit trees.

02 *ANTIMICROBIAL ACTIVITY OF SNAKIN-1 AND DEFENSIN-1 AS A HYBRID PROTEIN IN* IN VITRO *AND* IN VIVO *EXPERIMENTS AGAINST PLANT PATHOGENS*

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Phytopathogens attack a wide range of agriculturally important crops, causing significant yield losses. In recent years, antimicrobial proteins (AMPs) have been the subject of interest as primary candidates for plant protection applications since they exhibit a broad range of activity against bacteria, fungi, viruses and protozoa, low minimal inhibitory concentrations are required, a rapid mode of microbial cell damage occurs, and microorganisms do not acquire resistance to AMPs due to their mechanism of action.
In our work, to augment plant protection against phytopathogens, we constructed a fusion gene for the simultaneous expression of snakin-1 (SN1) and defensin-1 (PTH1) antimicrobial proteins as a hybrid protein (SAP), capable of proteolytic self-cleavage for coordinated production of the individual proteins in plant cells. Prior to *in vivo* evaluation of SAP phytoprotective activity, the hybrid protein expressed in *Escherichia coli* was tested against a variety of phytopathogenic microorganisms *in vitro*. SAP exhibited the highest antimicrobial activity against bacterium *Clavibacter michiganensis* subsp. *sepedonicus,* known to cause the potato ring rot disease, and anthracnose-causing fungus *Colletotrichum coccoides* by complete inhibition of cell growth or spore germination, respectively, at 6 μM concentration. Notably, the hybrid protein showed a

higher activity against *Pseudomonas syringae* pv. *syringae*, *P. syringae* pv. *tabaci* and *Colletotrichum coccoides*, than individual SN1 and PTH1, whereas its effect on *C. michiganensis* subsp. *sepedonicus in vitro* was comparable to that of PTH1 alone, indicating that in this case the activity was primarily determined by the PTH1 component of SAP. To evaluate antimicrobial properties of SAP *in vivo*, the gene coding hybrid protein was transiently expressed in plants from the *Potato virus X* (PVX)-based vectors (pP2C2S and pGR107). Expression of *sap* and genetic stability of the construct in plant tissues were verified by molecular assays. Antimicrobial activity of SAP against *C. coccoides* and *C. michiganensis* subsp. *sepedonicus* was assessed on *Nicotiana benthamiana* and *Solanum tuberosum,* respectively. Although no cleavage of SAP was observed in plant tissues, both *sap*-expressing plants were significantly more resistant to infection than plants in the control groups. Our results demonstrate that *in vivo* co-expression of recombinant *sn1* and *pth1* in the form of hybrid is a promising strategy for antimicrobial plant defense applications.

03 PROTEOMIC ANALYSIS OF ETHYLENE HORMONE SIGNALING IN Arabidopsis

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The plant hormone ethylene (C2H4) has profound effects on growth and development throughout the plant life cycle. Key components in the ethylene signal transduction pathway have been identified using molecular genetic approaches in the model plant *Arabidopsis thaliana*. However, our understanding of the ethylene signaling pathway remains incomplete, as the molecular mechanisms by which most of these components transmit the ethylene signal are unknown. For example the ethylene receptors and CTR1 are protein kinases, yet the potential substrates for phosphorylation have yet to be identified. Moreover, there are likely to be additional players in ethylene response that genetic screens have not uncovered. To address this, we have begun to investigate ethylene responses at the protein level by using liquid chromatography combined with tandem mass spectrometry (LC-MS/MS). Proteomics can potentially provide insight into ethylene signaling mechanisms while simultaneously identifying new components in the pathway. Protein was extracted from three-day old etiolated *Arabidopsis* seedlings treated with or without 100 μL/L ethylene for 3 hours. The membrane fraction was subjected to immobilized metal ion affinity chromatography to enrich for phosphopeptides, and the enriched phosphopeptide eluate and the non-phosphopeptide flow-through analyzed independently by liquid chromatography-tandem mass spectrometry (LC-MS/MS). Three pooled biological replicates were examined for changes in protein content and phosphorylation events, resulting in the identification of 5189 proteins in the membrane-enriched fraction of both ethylene-treated and untreated samples, with 2673 shared between them. Of the shared proteins, 185 showed significant accumulation changes after ethylene treatment and 708 of the 2673 shared proteins included at least one phosphopeptide. These data point to novel proteomic changes in the plant cell upon ethylene treatment. Preliminary data indicated that some interesting candidates may be involved in the ethylene response.

04 CYSTEINE MUTANTS OF MAIZE RAYADO FINO VIRUS-LIKE PARTICLES AS A PLATFORM FOR MULTIVALENT DISPLAY

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In recent years plant viruses have gained interest for nanotechnology applications including fabrication of biomaterials, drug targeting and vaccine delivery. Important characteristics of virus-based nanoparticle platforms (VNPs) include knowledge about their crystal structure, ability of many viruses to self-assemble into virus-like particles (VLPs), stability of VLPs at a wide range of pH and temperature conditions, and suitability for genetic manipulation as well as chemical bio-conjugation. Each subunit forming the VLP represents a scaffold to display peptides in a biologically functional form. This can be achieved by either genetically modifying the viral genome, or by chemically attaching *in vitro* synthesized peptides to reactive amino acids, such as cysteine (Cys), on the virus particles.

Maize rayado fino virus (MRFV) is the type member of the genus *Marafivirus*, family *Tymoviridae,* and is characterized by isometric T=3 particles about 30 nm in diameter. The viral capsid is composed of two components: empty shells, and complete virus particles, containing a single-stranded, monopartite, positive-sense RNA genome of 6.3 kb. The particles are composed of two serologically-related capsid proteins (CP) that, upon gel electrophoresis, exhibit a molecular mass of approximately 21–22 kDa (CP2) and 24–28 kDa (CP1); the two CPs contain common peptide sequences and are found in molar ratios of 3:1, respectively. MRFV CPs have been shown to self-assemble into VLPs (Hammond and Hammond 2010 Virus Res 147: 208) which could be used in a variety of nano- and biotechnology applications.

In the present report we evaluated the ability of plants produced MRFV-VLPs to serve as suitable scaffolds to which a variety of functional groups could be chemically attached on the exterior surface.

In order to provide an anchor for functional groups, Cys residues were substituted for several of the amino acids present on the surface of the wild-type virus particles producing mutants Cys-MRFV-VLPs. Three or five amino acids in three different predicted surface regions of the MRFV coat protein were selected for Cys substitution. Each selected amino acid was converted to Cys by site-directed mutagenesis. Consecutively, each CP1-mutant gene was cloned into a *Potato virus X* (PVX)-based vector to achieve high-level of plant-produced Cys-MRFV-VLPs. The mutants were screened by SDS gel electrophoresis and Western Blot analysis. The accessibility of free thiol- reactive groups on the shell of Cys-MRFV-VLPs mutants was also determined by their reaction under native conditions with the thiol-specific chemical reagents, fluorescein-5 malemide. These criteria enabled us to determine whether the mutants produced are intact and form properly folded CPs which present reactive Cys residues.

The ability to produce Cys-mutants of MRFV affords the possibility of producing a new platform for nanotechnology, molecular electronics, and biotechnology applications, including drug targeting and vaccine delivery. Further investigations are currently underway to explore these ***opportunities.***

05 *METHODS FOR INCREASING LEVELS OF TERPENOID ACTIVE INGREDIENT(S) IN BLACK COHOSH (ACTAEA RACEMOSA) AND ELUCIDATION OF THEIR ROLE IN PLANT DEFENSE*

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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 account 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. 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, ABA, hydrogen peroxide 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.

06 *GENETIC DIVERSITY AND SPATIAL STRUCTURE IN CACAO GERMPLASM FROM BOLIVIA*

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Cacao (*Theobroma cacao* L.) is an important economic crop widely cultivated in the Bolivian Amazon. The germplasm group used by the Bolivian farmers was called "Cacao Nacional Boliviano" (CNB). Wild cacao populations are also found in the Beni River and in the valleys of the Andes foot hills. Knowledge about the genetic diversity in this germplasm group and its relation to other Amazonian cacao populations is essential for understanding the distribution of the cacao gene pool in the Americas. Using DNA fingerprinting technology based on microsatellite markers, we genotyped 164 Bolivian cacao accessions, including both cultivated and wild CNB accessions sampled from the Department of La Paz and Beni and compared the SSR profiles with samples of reference accessions from the cacao gene pool in the Americas. Results of

multivariate ordination and analysis of molecular variance show that the Bolivian cacao has a unique genetic status that is significantly different from the known cacao germplasm groups in South America, including a neighboring Forastero population from the Peruvian Amazon (Fst=0.202; *P*<0.001). The results also show that the cultivated CNB share a similar genetic profile with the wild populations in the Beni River, suggesting that the cultivated CNB is indigenous to Bolivia. The level of genetic diversity, measured by allele richness and gene diversity in the Bolivian cacao is moderately high, but was significantly lower than that in the other upper Amazon cacao populations. Significant spatial genetic structure was found in the wild CNB population through the analysis of autocorrelation (rc=0.232; P<0.001). Mantel tests between genetic and geographical distances further support the pattern of isolation by distance in the wild CNB cacao (Rxy=0.276; P<0.001). These results, combined with the outcome of morphological characterizations, support the previous conclusion that the CNB is a group of Forastero cacao in the southwest extreme of the cacao gene pool in the Americas. This group represents a diversity gap that remains to be filled in for the International cacao gene banks.

07 IDENTIFICATION OF TRADITIONAL CACAO VARIETIES USING MICROSATELLITE AND SINGLE NUCLEI POLYMORPHISM (SNP) MARKERS

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Cacao (*Theobroma cacao* L.) is the main source for chocolate with an annual production of four million tons (2010). This Neotropical tree crop was domesticated in Mesoamerica as far back as 3,000 years ago. Traditional farmers' varieties, suitable for the production of fine flavor chocolate, still exist in farmers' fields in the Americas. However, these varieties are thinly scattered among ordinary bulk varieties and are being replaced by introduced exotic varieties. Accurate identification of these rare and valuable landraces is essential for sustainable production of fine- flavored cacao beans and contributes to *in situ*/on-farm conservation of these premium varieties. Using 15 microsatellite and 100 SNP markers, we genotyped 226 accessions collected from traditional farms in Central and South America. A total of 120 known accessions with population membership in the International Cocoa Gene banks were used as references. Cluster analysis and Bayesian assignment tests successfully identified traditional varieties based on their affiliation with known traditional cacao varieties, including 'Amelonado', 'Venezuela Porcelana', 'Comun', 'Criollo', 'Indio Rojo' and 'Nacional'. The molecular markers revealed inter-varietal relationships that are largely compatible with the geographic origin of these traditional varieties. The two marker systems were compared in terms of their genotyping efficiency, repeatability, allelic diversity and power for assessing mislabeling and redundancy. The analysis shows that high-throughput genotyping using a small set of SNP markers, combined with model based assignment test, is a cost-effective and accurate approach to meet the increasing demand to identify traditional cacao varieties.

08 ISOLATION OF TOTAL NUCLEIC ACID FOR MICROARRAY-BASED DETECTION OF VIRUSES IN PLANTS

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Globalization has increased the risk that damaging plant pathogens will be introduced or reintroduced into susceptible populations. Because imported plants and germplasm harboring infections may not present obvious disease symptoms, a rapid test to identity and categorize known and unknown plant pathogens is needed. 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. Three challenges to establishing this assay are: 1) design of oligonucleotide probes that detect and discriminate viral nucleic acids to the family and species level; 2) a method for prompt isolation of functional nucleic acids from any plant species; and 3) amplification and labeling of isolated viral nucleic acids to levels detectable by common microarray scanners. Secondary metabolites, such as polysaccharides and polyphenols, reduce the yield, quality, and functionality of nucleic acids isolated from many plant species. Because species vary in the makeup of secondary metabolites, published isolation methods often vary according to species and tissue. Furthermore, these methods are often time-consuming. Adapting techniques from multiple methods, we have developed a single protocol for isolating an acceptable yield of amplifiable total nucleic acid in two hours. Our CTAB-based protocol incorporates KOAc to reduce co-precipitation of polysaccharides and spin columns for increased purity and to reduce time needed for washing and resuspending nucleic acids. Using this protocol we have isolated total nucleic acid from a variety of plant families high in secondary metabolites such as polysaccharides, phenolics, and latex. Light absorption ratios show that isolated nucleic acids are more pure than those isolated by common methods, with most samples free of contaminants. Yields are sufficient for microarray experiments and RT-PCR confirms that isolated plant and viral nucleic acids are amplifiable. Current work is focused testing a broad range of plant species to confirm the universal application of the protocol. Also, we are transitioning to testing methods for total nucleic acid amplification and labeling suitable for detection by our universal plant virus microarray.

09 EXPRESSION ANALYSIS OF AN ALCOHOL OXIDASE GENE FROM THE FUNGUS *MONILIOPHTHORA RORERI*, THE CAUSAL AGENT OF CACAO FROSTY POD ROT

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Theobroma cacao is an economically important tree cultivated in tropical regions of the world. Its seeds are used to produce the cocoa and cocoa butter used in the production of chocolate. However, diseases attack the cacao crop in many producing countries, causing a decrease in cacao production, and generating big losses to the farmers and the chocolate industry. One of these

diseases is called the "frosty pod rot", caused by the basidiomycete fungus *Moniliophthora roreri*. After being infected by spores produced by this fungus, the cacao pods cannot be used in chocolate production due to the necrosis and rot symptoms caused by this disease. In order to understand the biology of *Moniliophthora roreri*, its genome is being sequenced by the Sustainable Perennial Crops Laboratory (USDA, USA) and the Expression and Genomics Laboratory (Unicamp, Brazil). One of the main goals of this genome project is to identify biochemical pathways used by this fungus during the interaction with cacao in order to develop control strategies to stop frosty pod rot. Alcohol oxidase is an enzyme that degrades alcohols, such as methanol, generating hydrogen peroxide and formaldehyde. A putative alcohol oxidase was found in the *M. roreri* genomic database. This gene is essential to the successful infection of many plant pathogenic fungi. The disruption of *Cladosporium fulvum* alcohol oxidase gene made this fungus unable to infect tomato plants. Our goal is to characterize *M. roreri* alcohol oxidase gene and its possible importance during the progression of frosty pod rot. Quantitative real-time PCR showed an 8-fold increase in the alcohol oxidase gene expression when the mycelium was grown in the presence of cacao extracts rather than glycerol. Its expression is also increased when the mycelia is grown in media with methanol or pectin as the sole carbon sources. The methyl chains present in the cacao pectin structure are probably the main source of methanol during the plant-pathogen interaction. In the next steps of this work, we intend to analyze the expression of *M. roreri* alcohol oxidase gene in planta during the progression of frosty pod rot and characterize this enzyme. The understanding of this biochemical pathway is important in the development of a biochemical model for this disease and future control strategies.

10 SOYBEAN RUST GENE EXPRESSION DURING APPRESSORIUM FORMATION

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Asian soybean rust (ASR), caused by the fungus *Phakopsora pachyrhizi*, has recently spread to North America. No current U.S. commercial soybean cultivars are resistant to ASR. Investigations into the molecular processes of the infection cycle may lead to the development of novel control strategies for this disease. *P. pachyrhizi* infects the host by direct penetration through the leaf cuticle. Direct penetration requires the formation of a specialized structure on the leaf surface called an appressorium. To examine gene expression during appressorium formation, an enriched cDNA library was constructed with mRNA extracted from appressoria produced by germinating urediniospores on polystyrene plates, and subtracted with mRNA extracted from urediniospores germinated on water. A total of 1152 cDNA clones were sequenced and compared to ESTs from *P. pachyrhizi* germinating urediniospores, and 31 clones were unique to the appressorium-enriched cDNA library. BlastX analysis revealed sequence similarity to known proteins for 20 clones, and identified three clones as hypothetical proteins. Eight clones showed no significant similarity to protein sequences in GenBank. Genes identified in this study fell into functional categories of metabolism, cell cycle and DNA processing, protein fate, cellular transport, cellular communication and signal transduction, and cell rescue.

11 *ANALYSIS OF* GLYCINE MAX *MRNA-SEQ DATA UPON A TIME-COURSE INFECTION WITH* PHAKOPSORA PACHYRHIZI

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mRNA-Seq has without a doubt, elevated to become a central tool in modern transcriptomics. Through this process, cDNA is sequenced of which can be used to quantify RNA expression. Numerous soybean pests exist, such as root-knot and soybean cyst nematode as well as soybean rust (*Phakopsora pachyrhizi*). The latter is an obligate fungus, exotic to the United States. In previous studies, we infected soybean with soybean rust, and utilized laser capture microdissection to isolate infection sites at different time-points along the infection process. We isolated and extracted RNA from infected sites, built cDNA libraries and employed RNA-Seq to sequence open reading frames of soybean (*Glycine max*). Our intent is to decipher the bigger picture regarding host-pathogen interactions. This study revealed a majority of induced genes were relative to plant-defense mechanisms, while most suppressed genes were associated with metabolism/biochemical synthesis. We aim to bring light into how such analysis was performed from a computational perspective, the various tools developed for this intent, and the biochemical pathways affected by this agricultural pest.

12 IDENTIFICATION OF DROUGHT RESPONSIVE GENES IN APPLE BY MODIFIED GENE SUBTRACTION

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Suppression subtractive hybridization (SSH) is a powerful tool for the global analysis of gene expression and has been used in this laboratory to identify drought-responsive genes and genes associated with drought recovery. Briefly, trees were subject to drought conditions for two weeks prior to mRNA isolation from leaf tissue. In a parallel experiment, trees that were subject to drought conditions were then re-watered for one week prior to mRNA isolation. cDNA was then synthesized according to standard procedures. Tester (experimental cDNA) and driver (reference cDNA) were hybridized in order to remove common sequences, after which the remaining cDNAs were amplified using PCR for subsequent analysis. Then, these two previously subtracted populations were used as tester and driver, respectively, in what might be thought of as a second-level subtraction using an extensively modified approach that was developed in this laboratory; the enriched, previously subtracted cDNAs were also hybridized against the original cDNA populations as well. This innovation facilitated the isolation of a subset of genes in apple, the expression of which correlates with drought recovery. These genes also differ from the genes that are up-regulated under well-watered growing conditions. The genes were then cloned, sequenced, and identified by BLAST analysis. The enriched subpopulation of genes identified by the application of this newly developed second-level subtraction methodology includes vitamin B12-independent methionine synthase, a metallothionein-like protein, and ubiquitin-activating enzyme E1. These genes have been shown previously to be associated with various forms of abiotic stress in apple and in several other plant species. The methodology described here may simplify the identification of master regulatory genes associated with the survival of plants under various forms of biotic and abiotic stress. This is a timely investigation because of the widespread occurrence of drought and the importance of apple as a commodity crop.

13 STRAWBERRY: A NEW MODEL FOR STUDYING FLOWER AND FRUIT DEVELOPMENT

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The diploid strawberry *Fragaria vesca* is an ideal model for developmental and genetic studies of the Rosaceae family, which includes the octoploid commercial strawberry (*Fragaria* x *ananassa*) as well as economically important fruits like apple, peach, and cherry. *F. vesca* can easily be grown in the lab, has a life cycle of only 3.5 months, can be propagated both sexually and vegetatively, does not produce runners, and is amenable to transformation. In addition, its 200Mb genome has been sequenced and is currently being assembled. Using a 7th generation diploid inbred line, 5AF7, we characterized the stages of strawberry flower development using scanning electron microscopy and histological sections. This morphological data will serve as the foundation for later developmental and genomic studies. Second, we are optimizing fixation protocols for tissues that will be dissected with laser capture microdissection (LCM) and used for reproductive tissue transcriptome analysis. Third, we are characterizing shoot and floral meristem architecture of *F. vesca* using both morphological and molecular markers. Finally, we have begun mutagenizing 5AF7 with several chemical mutagens to determine optimal mutagen treatment and to isolate developmental mutants. This work is supported by NSF (MCB0923913) to ZL and JS.

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