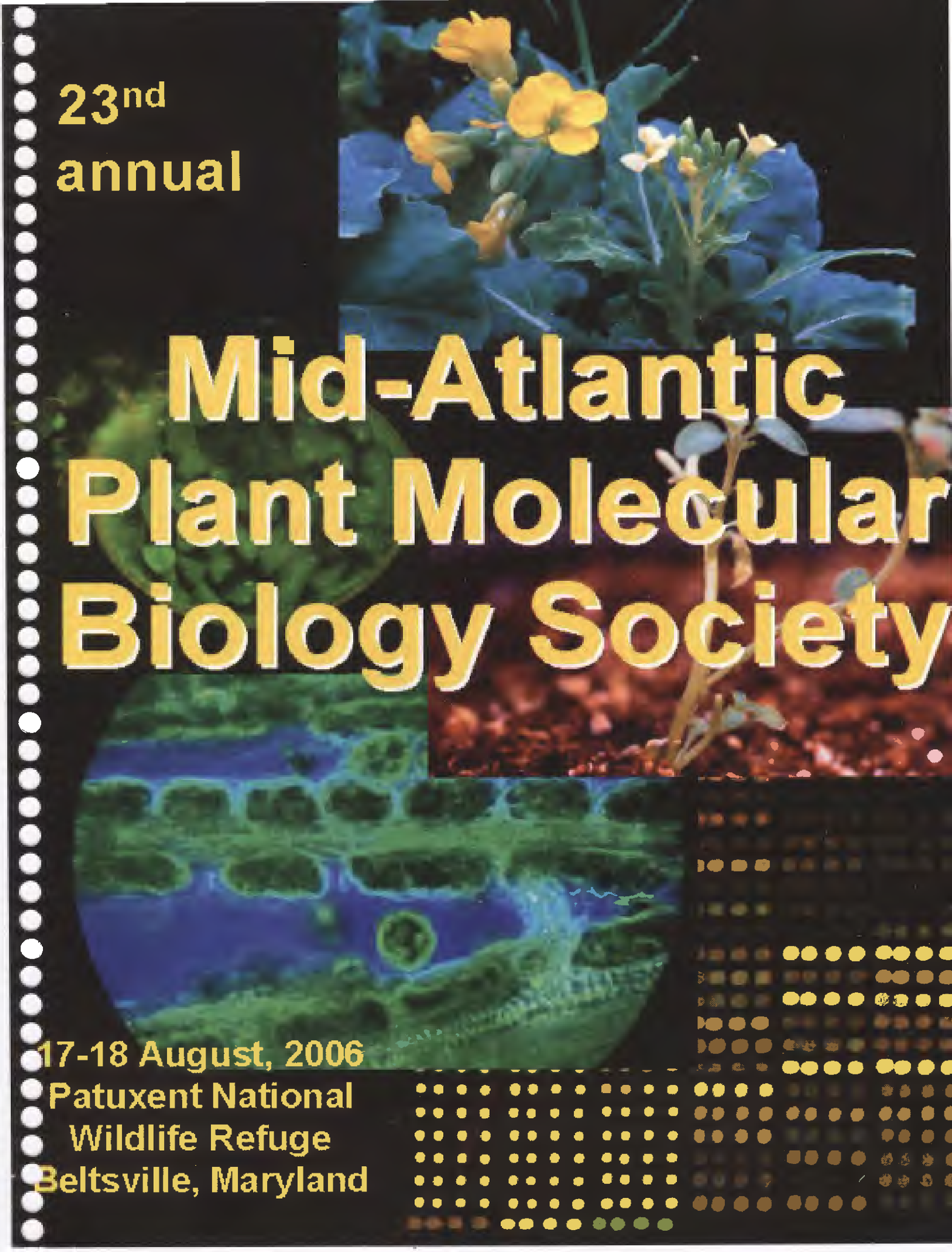

23nd annual

Mid-Atlantic Plant Molecular Biology Society

17-18 August, 2006
Patuxent National Wildlife Refuge
Beltsville, Maryland

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COVER DESIGN, ***Leslie Wanner, with photos supplied by Kristin Edwards, Jim Saunders and Ben Matthews***

INTRODUCTION

On behalf of the Organizing Committees we would like to welcome you to the twenty-second meeting of the Mid-Atlantic Plant Molecular Biology Society, MAPMBS 2006. The goal of this society is to provide a forum for area scientists to exchange ideas and information relating to plant molecular biology. This meeting is designed 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 at 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.

As you know, 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, you can join next years 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 thank our sponsors and exhibitors who furnish us with up-to-date products, and help to defray costs. Please be sure to visit our exhibitors in the multipurpose room.

We thank you for your continued support of and participation in the Mid-Atlantic Plant Molecular Biology Society.

Benjamin F. Matthews and Leslie A. Wanner
Co-Chairs

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2006 MAPMBS MEETING SCHEDULE

Thursday, August 17

9:00 Registration, poster set-up

9:25 Introductory remarks (Ben Matthews, USDA-ARS Soybean Genomics and Improvement Lab)

Session I Biotechnology and our Food Supply (Session chair: John Hammond, USDA-ARS Floral and Nursery Plants Research Unit*)*

9:30 Jed Fahey, Johns Hopkins University, ***"The Development of Cancer Chemoprotective Foods: A Journey Away from Academic Plant Science . . . and Back Again"***

10:05 Perry Cregan, USDA-ARS Soybean Genomics and Improvement Lab, ***"Domestication, founding effects and artificial selection - Genetic bottlenecks and soybean genetic variability and vulnerability"***

10:40 Poster Session, Coffee break, exhibitors

Session II Bioinformatics Tools (Session chair: Enno Krebbers, Dupont Crop Genetics Research, Wilmington, DE)

11:15 Habtom Ressom, Georgetown University, ***"Biomarker identification and sample classification using machine learning methods"***

11:45 Anna Maria Vaira, USDA-USNA Floral and Nursery Crops Research Unit, "***Diagnosis and characterization of Ophioviruses***"

12:05 LUNCH

Session III New technologies applied to understanding plant stresses (Session chair: Reid Frederick, USDA-ARS NAA Foreign Disease-Weed Science Research Unit*)*

1:20 Susan Lawrence, USDA-ARS "***Regurgitant from Colorado potato beetle inhibits tomato wound-induced defense genes"***

1:40 Jim English, University of Missouri, "***Peptide technologies for management of plant disease"***

2:10 Katherine Schneider, USDA-ARS Ft. Detrick, "***Microarray analysis of genes expressed in Rpp1-mediated resistance to Asian soybean rust"***

2:40 Coffee break, exhibitors

3:10 Introduction of Keynote Speaker, Brett Cooper, ***USDA-ARS Soybean Genomics and Improvement Lab***

3:30 Keynote Address: Phillip A. Rea, University of Pennsylvania, "How a synthetically active cysteine protease, phytochelatin synthase, comes to grips with heavy metals"

4:30 Close of Day

Friday, August 18

9:00 registration, exhibitors, posters

Session IV More on Plant Environmental Stress (Session chair: Sisir Dutta, Biology Department, Howard University)

9:30 Benildo de los Reyes, University of Maine, Orono, "***Assembling the cold stress transcriptional regulatory network of rice***"

10:00 Kristie Magee, Howard University, "***Bacteria and plant nitrate reductase gene-mediated dechlorination of hexachlorobiphenyl***"

10:20 Leisell Lashley, Howard University, "***Micobial community structure analysis of three PCB contaminated soils and alfalfa rhizospheres using high throughput 16s rDNA T-RFLP technology***"

10:40 Coffee Break, exhibitors and posters

Session V Bioinformatics Tools (Session chair: Brett Cooper, USDA-ARS Soybean Genomics and Improvement Lab)

11:10 Brett Tyler, Virginia Bioinformatics Institute, ***"Functional Genomics of the Phytophthora sojae-soybean Interaction"***

11:40 Business meeting

12:00 Lunch, exhibits

1:30 Hari Krishnan, University of Missouri, ***"Genetic modification of soybean seed composition"***

2:00 Hajime Sakai, Dupont Crop Genetics, ***"Forward genetics based gene discovery for maize ag traits***"

2:30 Vincent Klink, USDA-ARS Soybean Genomics and Improvement Lab, ***"Timecourse microarray analysis of Glycine max (soybean) roots infected by Heterodera glycines (soybean cyst nematode)"***

2:50 Close of day

THE DEVELOPMENT OF CANCER CHEMOPROTECTIVE FOODS: A JOURNEY AWAY FROM ACADEMIC PLANT SCIENCE . . . AND BACK AGAIN

Jed W. Fahey

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Over 120 glucosinolates have been identified in a large number of edible plant species. Much early research had focused upon the "antinutritional" or goitrogenic properties of some member of this diverse class of water soluble secondary metabolites which are abundant in crucifers and about 15 other plant families. Glucosinolates are enzymatically converted to isothiocyanates by myrosinase, an enzyme which coexists in the plant cells and is released upon damage to the plant tissue which contains them. Although their cancer-preventive potential of these isothiocyanates was initially described as a consequence of their induction of Phase 2 enzymes, they have more recently been shown to possess potent antiproliferative, apotosis-promoting, redox regulatory and phase 1 enzyme inhibitory roles, as well as being directly bactericidal against the carcinogenic bacterium, *Helicobacter pylori*. Since glucosinolates have potential value as phytochemical components of healthy diets, we have developed methods for enriching, isolating and purifying these compounds. We have also evaluated and developed germplasm enriched in certain glucosinolates. In addition to biochemical and molecular characterization of the mode of action of their active isothiocyanate metabolites, we have engaged in a variety of clinical trials designed to evaluate the protective effect of ingested glucosinolates on a number of diseases.

[*Dr. Fahey spent 15 years in the ag-biotech industry prior to going to Johns Hopkins in 1993. In the course of the academic research he will be describing, he co-founded a food company based on his discovery and development of broccoli sprouts as a potent source of chemoprotective glucosinolates. The MAPMBS organizers have asked that this journey be discussed in the course of the scientific presentation.*]

DOMESTICATION, FOUNDING EFFECTS AND ARTIFICIAL SELECTION - GENETIC BOTTLENECKS AND SOYBEAN GENETIC VARIABILITY AND VULNERABILITY

Perry B. Cregan[1], David L. Hyten[1], Qijian Song[1,2], Youlin Zhu[1,3], Ik-Young Choi[1], Randall L. Nelson[4], Jose M. Costa[2], and James E. Specht[5]

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Soybean has undergone several genetic bottlenecks. These include domestication in Asia to produce numerous Asian Landraces, introduction of relatively few Landraces to North America, and then selective plant breeding over the past 75 years. It is presumed that these three human-mediated events have reduced genetic diversity. We sequenced 111 fragments from 102 genes in four soybean populations representing the populations before and after genetic bottlenecks. We show that soybean has lost many rare sequence variants and had many allele frequency changes throughout its history. Although soybean genetic diversity has been eroded by human selection after domestication, it is notable that modern cultivars have retained 72% of the sequence diversity present in the Asian Landraces. Simulations indicated that the diversity lost through the genetic bottleneck of introduction and plant breeding was mostly due to the small number of Asian introductions, and not the artificial selection subsequently imposed by breeders. The bottleneck with the most impact was soybean domestication, when the low sequence diversity present in the wild species was halved and 60% of the genes exhibited evidence of significant allele frequency changes. The level of genetic vulnerability of a crop species is assumed to be inversely proportional to its genetic diversity. Genetic vulnerability is the condition of being broadly susceptible to attack by pests. The genetic vulnerability of the N. American soybean crop has been a longstanding concern that again became the focus of attention with the arrival of soybean rust in North America in 2004. The widely held assumption is that soybean breeding based upon a small number of founding Asian Landraces followed by the repeated use of a few elite cultivars in each breeding cycle has created a condition of substantial genetically vulnerability. Our data indicate that this is not the case. While genetic variability in North American soybean is low, this is not a result of recent human intervention but rather the intrinsically low variation in cultivated soybean. Our data also indicate that *G. soja* contains many unique alleles suggesting *G. soja* as the reservoir of rare disease resistance alleles needed to protect the soybean crop from new disease epiphytotics.

BIOMARKER IDENTIFICATION AND SAMPLE CLASSIFICATION USING MACHINE LEARNING METHODS

Habtom W. Ressom

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Lombardi Comprehensive Cancer Center
Georgetown University Medical Center

High-throughput technologies such as microarray and mass spectrometry generate a large amount of data, enabling molecular profiling of complex diseases such as cancer. Various computational methods have been proposed to analyze the high-dimensional data generated by these technologies for molecular classification of samples. However, most classification algorithms perform sub-optimally with thousands of features (genes/proteins). Thus, the selection of features (biomarkers) that are most predictive of a phenotype is required. This talk will present the use of machine learning methods for identification of candidate biomarkers that enhance the accuracy in molecular classification of samples.

DIAGNOSIS AND CHARACTERIZATION OF OPHIOVIRUSES

Anna Maria Vaira[1] and Hammond John[2]

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A new virus family, *Ophioviridae*, has been recently proposed to include the recently established genus *Ophiovirus*, showing peculiar characteristics. There are currently five species in the genus and at least one tentative species, all of them are plant-infecting viruses. The accepted species in the genus are: *Citrus psorosis virus* (CPsV), the type species, *Lettuce ring necrosis virus* (LRNV), *Mirafiori lettuce virus* (MiLV), *Ranunculus white mottle virus* (RWMV) and *Tulip mild mottle mosaic virus* (TMMMV). The Ophiovirus genome is ssRNA, 11.3-12.5 kb in size, divided into three or four segments (RNAs 1 to 4) and is considered negative sense. Positive-sense RNA is also encapsidated. The RNA dependent RNA polymerase is encoded by RNA 1 and the coat protein by RNA 3; no information is available regarding the synthesis or the function of the other ORF products. As the virions are not easily detectable with electron microscope techniques and serological cross-reaction of coat proteins is not consistent, RT-PCR amplification, using degenerate primers, of a 136 bp fragment from the RdRp gene, is currently the best tool for detecting and identifying species within the genus. Sequencing and phylogenetic analysis of the 45 amino acid string deduced from the amplified fragment of several isolates belonging to different species, not only fully supports the present species classification but has also been successfully used to obtain indication of the taxonomic positioning of newly diagnosed isolates.

An ophiovirus in freesia, provisionally named Freesia sneak virus (FreSV), considered a tentative species, was diagnosed and proposed as a new ophiovirus species owing to this procedure, when no other viral genome sequences were known; the complete coat protein sequence later obtained confirmed the hypothesis. A positive reaction with the genus-specific RT-PCR has recently been obtained for several samples from *Lachenalia*, a monocot in the family *Hyacinthaceae*; in this case the RT-PCR and subsequent analysis of the 45 amino acid string suggest placing the isolate within the Freesia sneak virus tentative species. Also in this case, subsequent coat protein gene sequencing, showed nearly 100% identity with the homologous portion of Freesia sneak virus at amino-acid level, confirming this position. Only three Ophiovirus isolates have been fully sequenced until now, and they are all dicot-infecting isolates. The genome of the *Lachenalia* isolate is currently under study using new degenerate primers for the cloning and sequencing procedure. The studies will provide information regarding the first genome sequence and organization of a monocot ornamental-infecting ophiovirus isolate.

REGURGITANT FROM COLORADO POTATO BEETLE INHIBITS TOMATO WOUND-INDUCED DEFENSE GENES

Susan D. Lawrence, Nicole G. Novak and Michael Blackburn

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One mechanism by which plants defend themselves against insect herbivores is the production of plant proteinase inhibitors, which can inhibit digestion in the midgut, thus affecting growth and survival. The effect of Colorado potato beetle (CPB) (*Leptinotarsa decemilineata* (Say) regurgitant on tomato, *Lycopersicon esculentum* defenses was investigated. When regurgitant from 4th instar CPB was applied to wounded tomato leaves, the wound-induced transcripts for the proteinase inhibitors *pin1* and *pin2* were reduced. Boiling the regurgitant abolished its ability to reduce the *pin* transcripts. Ultrafiltration of the regurgitant demonstrated that it contained a component between 10,000 and 30,000 MW that inhibited wound-induced *pin1* and *pin2* expression, suggesting that it may be a protein. This may represent a mechanism that the CPB has evolved to elude the plants induced response to infestation. Previous insect elicitors have been identified exclusively from caterpillars; this is the first example from beetles.

PEPTIDE TECHNOLOGIES FOR MANAGEMENT OF PLANT DISEASE

James T. English

Division of Plant Sciences, 108 Waters Hall, University of Missouri, Columbia, MO 65211

Agricultural crops are under constant challenge from emerging and indigenous pathogens. In many cases, there is limited resistance available in germplasm of a specific crop for plant protection. There are also limitations in availability of genomic and proteomic resources for many important plant pathogens. In response to this situation, we have developed an approach for selective plant defense that does not require genomic or proteomic profiles of host-pathogen interactions.

The strategy is based on the identification of small, combinatorially selected peptides that function as inhibitory ligand mimics for pathogen cell-surface factors involved in growth and development. The three steps of this strategy include (1) selection of peptides with high affinity for plant cell-surface receptors, (2) assessment of impacts of affinity-selected peptides on pathogen development and, (3) delivery of bioactive peptides in susceptible tissues of host plants.

We have applied this strategy successfully to limit tomato root infection by the oomycetous pathogen, *Phytophthora capsici*. Peptides, selected from combinatorial phage-display libraries, were chosen for their ability to induce premature zoospore encystment and thus block chemotactic movement to root surfaces. These bioactive peptides were fused to a naturally occurring, secretable plant protein that serves as a scaffold for peptide display. When expressed in tomato roots, scaffold-peptides were secreted from roots, blocked zoospore chemotaxis and reduced root infection.

We have recently applied these methods to identify peptides that halt the growth and development of bacterial and fungal pathogens of plants and humans. We expect that this peptide strategy will be useful in developing defense responses against pathogens that appear unexpectedly in a region and in response to new forms of pathogen resistance as they develop in nature.

ANALYSIS OF GENE EXPRESSION IN RPP1-MEDIATED RESISTANCE TO ASIAN SOYBEAN RUST

Katherine T. Schneider[1], Jane J. Choi[1], Nadim W. Alkharouf[2], Nicole L. Lum[3], David J. Munroe[3], Benjamin F. Matthews[4], and Reid D. Frederick[1]

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[4]Soybean Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD 20705

Asian soybean rust caused by *Phakopsora pachyrhizi* is native to Asia, and has spread to Africa, Australia, South America, and Hawaii. In November 2004, soybean rust was identified for the first time in the continental U.S. Soybean rust is of particular concern because of its potential to reduce yields and increase production costs for U.S. growers. While no known rust resistance exists in commercially available U.S. soybean cultivars, four independent resistance genes (*Rpp1-Rpp4)* have been described that recognize specific rust isolates. To identify additional genes that contribute to the *Rpp1*-mediated resistant reaction, a soybean-rust cDNA library enriched for resistance-related transcripts was constructed using suppressive subtractive hybridization (SSH). The SSH cDNA library showed an enrichment of soybean cDNA clones that were placed into functional categories of cell rescue/defense/stress (6%), cellular transport (11%), protein fate (7%), and others. Gene expression was compared between *Rpp1* resistant and susceptible rust interactions using two soybean microarrays: 1) a 7883 soybean cDNA clone microarray including the clones from the SSH enriched library, and 2) the Affymetrix Soybean Genome Chip. Clones with similarity to peroxidases and lipoxygenases were up-regulated, as were putative anti-apoptosis genes. Down-regulated cDNA clones included those with similarity to cell wall-associated proteins and proline-rich proteins.

HOW A SYNTHETICALLY ACTIVE CYSTEINE PROTEASE, PHYTOCHELATIN SYNTHASE, COMES TO GRIPS WITH HEAVY METALS

Philip A. Rea

Plant Science Institute, Department of Biology, Carolyn Hoff Lynch Biology Laboratory, University of Pennsylvania, Philadelphia, PA 19104-6018

Supraoptimal levels of essential heavy metals and trace or higher levels of nonessential heavy metals, as exemplified by copper and zinc, and arsenic, cadmium, lead and mercury, respectively, undergo aberrant capping reactions with the thiol groups of proteins and some thiol-containing coenzymes, displace endogenous metal cofactors (heavy or otherwise) from their cellular binding sites and promote the formation of destructive active oxygen species. Of the several mechanisms known to confer protection from such adversity, one of the most sophisticated is the enzyme-catalyzed synthesis of mixed γ- and α-peptidyl polymers, phytochelatins (PCs), by vascular plants and some algae, diatoms, fungi and invertebrates, when exposed to cadmium, arsenic and its oxides, or mercury. PCs, which serve as high-affinity, thiol-rich cellular chelators and contribute to the detoxification of heavy metal ions, notably Cd^{2+}, have the general structure $(\gamma\text{-Glu-Cys})_n$-Xaa, where $n = 2\text{-}11$ and Xaa is usually Gly, are derived from glutathione (γ -Glu-Cys-Gly) and related thiols in a reaction catalyzed by phytochelatin synthases (PC synthases; EC 2.3.2.15). In this talk, an account will be provided of how through the application of relatively straightforward biochemical and bioinformatic approaches in combination with simple molecular manipulations the core catalytic mechanism of PC synthases has been elucidated using the enzyme from *Arabidopsis* (AtPCS1) as a model. It will be explained how it was inferred that: (i) the enzyme is a γ -Glu-Cys dipeptidyl transpeptidase that mediates a bisubstrate reaction in which the thiol group(s) of at least one of the substrates is(are) blocked, usually but not necessarily through the formation of a heavy metal thiolate; (ii) the reaction approximates ping-pong kinetics and is strictly associated with γ -Glu-Cys acylation of the enzyme at two sites, one of which corresponds to or is closely coupled to AtPCS1 Cys^{56}, or its equivalents in other PC synthases; (iii) the N-terminal domain of eukaryotic PC synthases, the portion that is represented in the half-molecule homologs from prokaryotes, is responsible for core catalysis through deployment of a cysteine protease-like Cys-His-Asp catalytic triad; (iv) heavy metals are not crucial for core catalysis by the N-terminal domain of AtPCS1, other than through substrate thiolate formation, although they are capable of augmenting synthetic activity through direct interaction with the C-terminal domain, a domain peculiar to eukaryotic enzymes. In short, the speaker will step by step present the logic and experiments behind the conclusion that AtPCS1, and by implication other eukaryotic PC synthases, is a novel synthetically-biased papain cysteine protease superfamily member that has (unlike its prokaryotic counterparts) an auxiliary metal-sensing C-terminal domain that undergoes secondary γ -Glu-Cys acylation, as well as a papain-like N-terminal catalytic domain that undergoes primary γ -Glu-Cys acylation.

Work from the Rea laboratory was supported by the United States Department of Agriculture National Research Initiative, Department of Energy, and National Science Foundation.

ASSEMBLING THE COLD STRESS TRANSCRIPTIONAL REGULATORY NETWORK OF RICE

Benildo G. de los Reyes

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Transcriptional regulation is an important aspect of the complex network of genes involved in plant responses to low temperatures. In cold-acclimating plants, the centerpiece of such network is the CBF/DREB family of transcription factors. In non-acclimating plants like rice, the nature of such network is not well understood. This study explores the wide differences in cold-sensitivity between indica and japonica and the available genomics resources for rice as a means to dissect the transcriptional regulatory pathways of plants that do not cold-acclimate. This goal was addressed by reconstructing the hierarchical organization of the various 'regulator-effector' modules of gene expression that are associated with adaptive responses. A snapshot of the early branch of the network was revealed by a set of data generated from the integrative analysis of the sequential trends in gene expression and conserved structural features in the promoters of co-regulated genes using the japonica map-based genome sequence as model.

The transcriptome profile of the cold-tolerant japonica rice (CT6748-8-CA-17) during the initial 24 hours of stress (10°C) showed that the early response involves two waves of induction. The first started within the first 2 hours of stress, i.e., rapidly induced early response genes (Group-I). The second did not start until after 2 hours of stress, i.e., delayed induced early response genes (Group-II). A number of transcription factors were among the genes that clustered under Group-I, which includes a homolog of the *CBF1* (*OsCBF1*) and a novel bZIP protein (OsbZIP-RLT) with similarity to some of the members of the class of bZIP proteins associated with the regulation of vascular tissue development (Class-i). Both transcription factors exhibited genotype-specific expression signatures, which is characterized by early and robust induction in cold-tolerant (CT6748-8-CA-17 and Nipponbare) but not in intolerant (INIAP12) rice genotype. Group-II genes were clustered according to the type of cis-elements that are represented in their promoters. The largest two include a cluster with CRT/DRE and/or ABRE and another cluster with the as-1/ocs-like and/or Myb-like sequences as their signature cis-elements. Deletion of the promoters of representative Group-II genes from each cluster and results of transgenic overexpression of the candidate regulators were consistent with the synchronized expression patterns of putative target clusters and their respective activators observed in the microarray and qPCR experiments. These results provide a partial definition of the composition of the early response transcriptional regulatory networks associated with genotypic differences in cold-sensitivity between japonica and indica rice. Insights on the possible differences between the Arabidopsis (long-term adaptation) and rice (short-term adaptation) regulatory networks will be discussed.

BACTERIA AND PLANT NITRATE REDUCTASE GENE-MEDIATED DECHLORINATION OF HEXACHLOROBIPHENYL

K. Magee, H. Ullah, and S. K. Dutta

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Introduction: Although once widely used in industry, polychlorinated biphenyls (PCBs) lost their popularity and became notorious for the deleterious effects that they can cause. Such effects range from neurotoxicity, endocrine disruption and reproductive abnormalities, to cancers. Strategies to eradicate the phenyl-ringed compound have been employed while the search for new and better strategies continues. PCB degradation typically occurs through an initial reductive dechlorination under anaerobic conditions followed by phenolic ring cleavage under aerobic conditions. Our laboratory has recently provided evidence that the white-rot fungus *Phanerochaete chrysosporium*, which has been shown to possess a nitrate reductase (NaR) gene, has the ability to degrade PCB aerobically under non-lignolytic (high nitrogen) conditions. While some fungi and bacteria are able to express NaR, plants primarily express this enzyme either constitutively in very low levels or upon induction by light and nitrate, the substrate. ***Hypothesis:*** The inducible nitrate reductase gene(s) of plants is able to reductively dechlorinate PCB under aerobic conditions. ***Methodology:*** Preliminary data using different types of bacteria was initially acquired before proceeding to plant work. For plant work, mature alfalfa was subjected to 24 hours of light in the presence and absence of nitrate. The leaves (2g) were subsequently harvested and ground using a grinding buffer (4ml). The final extract sample was used for the following *in vitro* enzyme reactions: (1) ***50uL extract*** with NADH, (2) ***15uL extract*** with NADH, (3) ***no extract*** with NADH, and (4) 50uL extract, ***no NADH***. PCB-153, standard assay solution, and in some cases, grinding buffer and water, were added to all reactions for a final volume of 0.5 mL. Incubation occurred for 10 minutes at 30°C and pH 7.5. To determine the activity of NaR in *Medicago sativa*, nitrite release assays were done. The dechlorination of PCB-153 was observed through several analyses: Gas Chromatography-Mass Spectroscopy (GC-MS), chlorine release assays, and Fourier-Transformed Infrared Spectroscopy (FTIR). For further confirmation, the dechlorination capability of a pure NaR enzyme from *Zea mays* (Sigma) was also analyzed by FTIR. Based on a partial cDNA sequence of *Medicago sativa's* NaR gene in GenBank and a highly conserved sequence of *Glycine max* NaR, attempts are underway to clone the *Medicago sativa* full-length NaR cDNA. A real-time PCR technique is being used to investigate the gene expression pattern under diverse physiological conditions. ***Results:*** Nitrite release data revealed the customary pattern of NaR activity: Activity of the enzyme is greatest in the presence of nitrate as well as NADH. For GC-MS analysis, biphenyl (BPH) was used as an internal standard, and the biphenyl and hexachlorobiphenyl peaks appeared at ~6.83 and ~10.14, respectively. To assess the degradation of PCB, PCB/BPH ratios were obtained according to area percentage of the peaks. In nitrate-induced plants, peaks for reaction 1 were unidentifiable. PCB/BPH ratios for reactions 2, 3, and 4 were as follows: 1.921, 1.271, and 0.341. In plants not induced by nitrate, PCB/BPH ratios for reactions 1, 2, 3, and 4 were 1.024, 0.872, 0.623, and 0.798, respectively. Chlorine release data generally correlates to the GC-MS results. For FTIR analysis of *Zea mays* NaR, PCB-153 was dissolved in methanol at 1,000 ppm to identify the C-Cl region; the C-Cl region appeared at 820.31 cm^{-1} and became a standard for all other spectra. In control samples devoid of the NaR enzyme, the C-Cl peak was evident at 830.48 cm^{-1} and had an absorbance of 0.68 while samples containing the NaR enzyme had a C-Cl peak at 823.68 cm^{-1} with an absorbance of 0.56. The addition of NaR's cofactor, molybdenum, caused a greater decrease in the C-Cl peak from 0.56 to 0.15. ***Discussion***: The results involving *Medicago sativa* support our overall hypothesis that inducible nitrate reductase gene(s) of plants is able to reductively dechlorinate PCB under aerobic conditions. In experiments where plants were induced by light and nitrate, greater amounts of the enzyme extract showed greater degradation of PCB. Significant degradation of PCB managed to occur without NADH, the electron donor, even though having this donor did improve degradation. In experiments where plants were induced by light but not by nitrate, no particular pattern for the degradation of PCB occurred. *Zea mays* experiments further support our hypothesis and suggest that the cofactor of NaR enhances dechlorination. These findings are significant as they may be applied to PCB bioremediation efforts. Supported in part by grants from the EPA and the NIH to S.K. Dutta.

MICROBIAL COMMUNITY STRUCTURE ANALYSIS OF THREE PCB CONTAMINATED SOILS AND ALFALFA RHIZOSPHERES USING HIGH THROUGHPUT 16S rDNA T-RFLP TECHNOLOGY

Leisel A. Lashley[1], Sue Mischke[2], Dapeng Zhang[2], Clayton Rugh[3], John Tharakan[1] and Sisir K. Dutta[1]

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INTRODUCTION: Previous studies in our laboratories suggest that under PCB-contaminated conditions, alfalfa can provide favorable soil conditions for soil rhizospheric microflora activity. PCB-dechlorinating soil bacteria are able to use plant rhizosphere substrates as a nutrient source allowing them to proliferate in the site. The environmental need for this investigation is to formulate a high-throughput process that would allow monitoring and subsequent optimization of bioremediation practices at these contaminated sites. Recent molecular applications in 16S rDNA microbial community profiling refer to T-RFLP (Terminal Restriction Fragment Length Polymorphism) as a high-throughput technique. ***HYPOTHESIS:*** 16S rDNA high-throughput T-RFLP advanced technology enables rapid identification and community structure analysis of polychlorinated biphenyl (PCB) degrading microbes in soils and alfalfa plant rhizospheres. ***MATERIALS AND METHODS:*** This study involved the quantitation of soil PCB concentration using HPLC. Soil DNA extraction was done using Mobio Ultraclean kit, and alfalfa plants for experimental rhizosphere treatment were used. Purified fluorescently-labeled amplicons from PCR reactions were digested with diagnostic restriction enzymes in separate reactions to distinguish representatives of the total bacterial community. A CEQ 8000 Genetic Analyzer was used to identify fragments for community structure analysis. The Ribosomal Database Project (RDP) web-based program was used for the 16S rDNA comparative microbial community designations. The RDP database contains almost 2,900 aligned 16S rDNA sequences for T-RFLP comparisons. ***RESULTS:*** Quantitative analyses of PCB contamination at two Superfund sites revealed PCB concentration of ~7-10ppm, Kalamazoo, Michigan (MI), and TSCA (Toxic Substance Control Act – 1976) Gary, Indiana (IN), having 1000ppm. Plate count assays were used, to observe $53x10^6$ CFUs/g in the MI rhizosphere soil and $20x10^5$ CFUs/g in the IN rhizosphere soil. Qualitatively, each T-RFLP pattern constitutes a distinct fingerprint of a community based on the number and molecular weight of T-RFs and abundance reflected by the size of each peak in the electrophoregram. *HhaI* restriction enzyme produced peaks at 68, 154, 206, 222 and 333 (lengths in base pairs), while *RsaI* yielded 118 and 121 peaks. The peaks at 68 and 118 for the IN TSCA site soil extracts most closely correlate to *Zooglea* genera. *Zooglea* genera have long been considered to be a typical activated sludge bacterium. ***DISCUSSION AND CONCLUSION:*** The expected environmental benefit would be to use the T-RFLP profiles to quantify the bacteria in all contaminated soils and effectively indicate the champion PCB degrader(s) in the presence of Alfalfa rhizospheres for bioremedial practices. T-RFLP, in the analysis of high to low PCB contamination - in the presence and absence of alfalfa rhizospheres - can compare and contrast microbial community structures of PCB-contaminated sites. Supported partially by grants from EPA and NIH to S. K. Dutta.

FUNCTIONAL GENOMICS OF THE PHYTOPHTHORA SOJAE-SOYBEAN INTERACTION

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Oomycete plant pathogens such as *Phytophthora* species and downy mildews cause destructive diseases of an enormous variety of crop plant species as well as forests and native ecosystems. These organisms are most closely related to algae in the kingdom Stramenopiles, and hence have evolved plant pathogenicity independently of other plant pathogens such as fungi. We have used structural genomics (genome sequence comparisons) and functional genomics (transcriptional profiling) to identify plant and pathogen genes that may be key players in the interaction between the soybean pathogen *Phytophthora sojae* and its host. In *P. sojae*, we have identified many rapidly diversifying gene families that encode potential pathogenicity factors including protein toxins, and a class of proteins (avirulence or effector proteins) that appear to have the ability to penetrate plant cells. Transcriptomic analysis of quantitative or multigenic resistance against *P. sojae* in soybean has revealed that there are widespread adjustments in host gene expression in response to infection, and that some responses are unique to particular resistant cultivars. These observations lay the foundation for dissecting the interplay between pathogen and host genes during infection at a whole-genome level.

GENETIC MODIFICATION OF SOYBEAN SEED COMPOSITION

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At present the economic value of soybeans is based principally upon the protein and oil content of the seed. Increasing competition from other oil and protein producing crops requires us to focus on methods of enhancing the quantity and quality of these key soybean seed components. Our laboratory is focused on modification of traits that will improve soybean seed composition, ultimately for the benefit of US soybean growers. One area of research deals with the improvement of sulfur amino acid content of soybean seed proteins. We have successfully generated transgenic soybean plants that accumulate zein, a methionine-rich maize protein. Even though seed-specific accumulation of zein was seen in soybean, the overall sulfur amino acid content was not enhanced. Interestingly, when the transgenic soybeans were grown in presence of supplemental sulfur, accumulation of the zein was elevated suggesting that the availability of sulfur is not adequate to meet the demand created by the introduction of a methionine-rich protein. To increase sulfur availability to developing seeds, we are currently producing transgenic soybean plants over expressing *O*-acetylserine (thiol) lyase (OASTL) and serine acetyltransferase (SAT), two key enzymes in the sulfur assimilatory pathway. Research is also being conducted to produce soybeans with high sucrose content by diverting the fixed carbon to the synthesis of sucrose instead of starch. We have generated transgenic soybeans in which expression of ADP-glucose pyrophosphorlyase (AGPase), a key regulatory enzyme in the starch biosynthetic pathway, has been silenced by RNAi. Constitutive silencing of AGPase resulted in a significant increase in the sucrose content of leaf tissue of transgenic soybeans. High sucrose content will enhance the quality of tofu and improve the palatability of edamame soybeans, which are a dietary staple in Asian countries.

FORWARD GENETICS BASED GENE DISCOVERY FOR MAIZE AG TRAITS

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In the last decade, genomics provided us with an enormous amount of information on genes, their genomic organization and expression patterns. Sophisticated genomics tools led us to candidate genes that appear to correlate with certain phenotypes and traits. The ways how these analyses are performed are convoluted and often inconclusive to draw a picture of genes required and sufficient for certain functionality. In order to bridge the gap, we are focused on forward genetics approaches, first finding genetic variants with phenotypes of interest and then identifying the causal genes. In recent years, map-based cloning became more and more feasible in maize. I would like to give two examples of our success taking this approach, one is the cloning of a maize root architecture gene, *RTCS* and another is of *RCG1*, a QTL gene conferring stalk rot resistance.

TIMECOURSE MICROARRAY ANALYSIS OF GLYCINE MAX (SOYBEAN) ROOTS INFECTED BY HETERODERA GLYCINES (SOYBEAN CYST NEMATODE)

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The soybean cyst nematode (SCN), *Heterodera glycines*, is the major pathogen of soybean (*G. max*), causing an estimated one-half to one billion dollars in damage each year in the U.S. We have been studying the *G. max-H. glycines* host-pathogen interaction through a variety of means. *G. max* roots susceptible and resistant to *H. glycines*, were inoculated and allowed to develop feeding sites (syncytia). We identified and isolated numerous *G. max* genes that are both induced in resistant plants and suppressed in susceptible plants during *H. glycines* infection. We have also identified developmentally regulated *H. glycines* genes that may act during important aspects of its infection.

01 GENERATING A LOW PHYTIC ACID SOYBEAN: TARGETING INOSITOL KINASE ENZYMES IN GLYCINE MAX

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Phytate (*myo*-inositol hexakisphosphate) is the major storage form of phosphorus present in plant seeds. Phosphorous in this form is indigestible by non-ruminant animals. Phytate molecules also bind essential mineral nutrients such as calcium, iron, and zinc, rendering them unavailable and classifying phytic acid as an anti-nutrient. Phytate in livestock feed contributes to a lower nutrient value, and the excreted indigestible phytate can lead to phosphorus pollution and eutrophication of water systems.

Several *low phytic acid* (*lpa*) mutant crop species have been developed, and livestock feeding studies with these mutants have demonstrated reduced levels of excreted phosphorous and improved mineral availability and uptake as a result of their incorporation into feed. However, all current *lpa* crop species have been created through random mutagenesis and generally carry yield penalties. Little is known regarding the biosynthetic pathway of phytic acid in plants or the secondary affects caused by mutations in the pathway. Altering seed composition to reduce phytate, improve nutrient availability, and reduce phosphorus pollution will require an understanding of the phytate biosynthetic pathway.

In this study, I am identifying and characterizing key enzymes in the phytic acid biosynthetic pathway, with the long term goal of developing soybean plants with silenced copies of specific biosynthetic genes. We have isolated several *myo*-inositol phosphate kinase genes in soybean as possible candidates for steps in the biosynthetic pathway. We have characterized the genes for four *myo*-inositol (1,3,4)P_3 5/6-kinases, one *myo*-inositol(1,4,5)P_3 6/3-kinase, and one *myo*-inositol(1,3,4,5,6)P_5 2-kinase. We have examined expression in developing seeds and other tissues by Northern blot analysis and quantitative RT-PCR. We have expressed all six genes as GST fusion proteins in *E. coli*, and verified enzyme activity on the proposed substrates. We are conducting biochemical characterization to determine enzyme kinetics and substrate specificities. We are utilizing soybean embryogenic cultures to test for alterations in inositol phosphate profiles as a result of down-regulating the kinase genes by RNA interference.

02 STUDIES OF ANTHOCYANIN REGULATORY GENES IN PHALAENOPSIS

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Anthocyanin biosynthesis is regulated at the transcription level and is generally controlled by two families of regulatory factors, R/B-like MYC and R2R3-MYB proteins. We first confirmed that the albescent phenotype (white petals, colored labellum) in *Phalaenopsis amabilis* could be complemented by transient expression of maize *Lc* (*myc*) and *C1* (*myb*) regulatory genes while neither *Lc* nor *C1* alone could induce anthocyanin production in the colorless petals. To further our understanding of the *myc* and *myb* differential transcription between the anthocyanin-defective and anthocyanin-productive *Phalaenopsis*, we made use of the highly conserved MYC domain (basic helix-loop-helix) and MYB domain (helix-turn-helix) and designed primers flanking these regions, with expected lengths of about 400 and 180 bps. The cDNA fragments produced by RT-PCRs from RNAs extracted from petals of *P. amabilis* (white) and *P. schilleriana* (magenta) were cloned and sequenced. For the *myc* gene, we sequenced 8 randomly selected clones, two of which were from colored petals. There were no hits with a blastn search, but a blastX search showed highly homologous matches (E>1e-30) to a *Cornus* myc-like anthocyanin regulatory protein. Five of the clones that had an insert of 411 bp displayed four SNPs, two of which caused nonsynonymous changes. The other three clones contained various deletions up to 100 bp. While one of the two clones derived from the colored petals was identical to a clone from the colorless petals, the other clone was quite different in that it had a 101 bp deletion at the 3' end of the clone and was markedly different in the remaining aligned region. We intend to isolate the full length copy of these putative *myc* genes to study their effect on anthocyanin expression.

03 ACTIVATION MECHANISM FOR A BINUCLEAR ZINC TRANSCRIPTION FACTOR IN A NOVEL FUNGAL STRESS PATHWAY

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Pathogens respond to stress conditions by activating signal transduction cascades, ultimately leading to specific gene expression to overcome the stress causing factors. *Fusarium solani* is a pathogenic filamentous fungus which causes root rot in pea. The fungus responds to a plant defense compound, pisatin, uniquely produced by the pea plant. The defense response is mediated by the activation of a fungal transcription factor, PRF (Pisatin Response Factor). Evidence from our experiments in a related fungus *Neurospora crassa* suggests that PRF might be involved in a common stress pathway as opposed to a compound specific response. I am studying the mechanism by which PRF functions in the cell. One approach I have used is yeast two hybrid analysis to identify other proteins with which PRF might interact in the cell. One of the proteins identified from the screen, V25, has a mitochondrial targeting sequence and is similar to the c terminal domain of superoxide dismutases. In order to understand the functional significance of the interaction, I have knocked out the *N.crassa* homologue of the interacting protein. Introducing PRF in this strain will allow me to study any change in the induction pattern in response to stress signals. The V25 homologue seems to be an essential gene as attempts to knockout the gene has produced recombinants with either the native gene alone or with both the marker gene and native gene. In a second approach, I am trying to characterize the size and nature of PRF in the cell by doing Western blots using anti-PRF antibody. Western blots with the purified antibody identified different sized forms of PRF. Treatment with phosphatases shifts the mobility of some bands, indicating phosphorylation. As a third approach, I have isolated multiple cDNA clones and find evidence for alternate splicing. Such alternate splicing may be involved in regulation of PRF activity. We are now studying the relevance of these three different possibilities in pisatin regulation of PRF.

04 HAEMOPOIETIC CANCERS AND THE HEDGEHOG PATHWAY

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There is growing evidence that specific genes and pathways required for cell growth and differentiation during development of the normal embryo play an important role in the development of human cancers. We have been studying one such pathway, the Sonic Hedgehog signaling pathway in cancers that affect the bone marrow and blood [1]. The hedgehog (Hh) gene was originally found to be required for the proper patterning of the fruitfly, *Drosophilia melanogaster,* and is very important in the development of the eye, wings, and brain. Response to the Hedgehog signal is controlled by two transmembrane proteins, the membrane bound receptor Patched (PTCH), and the proto-oncogene Smoothened (smo) [2]. During normal pathway activation, Hh binds to Ptch thus alleviating suppression of Smo. Smo signaling triggers a cascade of intracellular events, leading to activation of the pathway through GLI-dependent transcription. We found that the Hh pathway is over active in human acute leukemias, and inhibition of the pathway leads to the death of tumor cells. We hypothesize that the understanding of Hh will lead to a better understanding of how cancers, such as leukemia, arise and the development of new therapies for these diseases.

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05 MAPK SIGNALING IN PTO-MEDIATED RESISTANCE

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We study the molecular interactions that govern resistance in tomato leaves towards *Pseudomonas syringae* pv. *tomato,* the causative agent of bacterial speck disease. During infection, *P. s.* pv. *tomato* translocates proteins into host cells by the bacterial type III secretion system. The recognition of two of these proteins, AvrPto and AvrPtoB, activates defense responses in the host, which culminate in cell death at the site of infection and limit growth and spread of the pathogen. Recognition of both AvrPto and AvrPtoB is mediated by the serine/threonine protein kinase Pto (1). Through a combination of virus-induced gene silencing, biochemical, and immunological methods we have demonstrated that mitogen-activated protein kinases (MAPKs) play an important role in the signaling events required for defense towards *P. s.* pv. *tomato* (2, 3, 4). We have shown that two MAPKs, LeMPK2 and LeMPK3, are activated in tomato leaves in a Pto-dependent manner following the recognition of AvrPto or AvrPtoB (2). Two phylogenetically unrelated MAPK kinases (MAPKKs), LeMKK2 and LeMKK4, activate LeMPK2 and LeMPK3 when overexpressed in leaf tissue, prior to the onset of cell death. LeMKK2 and LeMKK4 phosphorylate LeMPK2 and LeMPK3 *in vitro* (2). Although the function of LeMKK4 is not known, LeMKK2 is required for Pto-mediated resistance (3, 4). A MAPKK kinase, LeMAPKKK□, functions upstream of LeMKK2 and plays an important role in the regulation of cell death in disease resistance and in susceptible leaves during infection (3). We are currently working to biochemically characterize LeMAPKKK□ and to identify other proteins that contribute to its function.

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06 PREDICTABLE TRANSITIVE RNA INTERFERENCE INDUCED BY MRNA HAIRPINS IN C. ELEGANS

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The specific silencing of genes through RNA interference (RNAi) by double-stranded RNA is now being studied not only as a means to understand cellular processes, but also as a means of treating patients in the pharmaceutical industry and controlling pests in the agricultural sector. While the protein machinery necessary to cause this phenomenon has been well characterized, it is not always possible to predict the silencing of one specific gene. At times, when a target gene is silenced, it can lead to the silencing of other RNAs. We are trying to understand this process, termed transitive RNAi, and trying to determine if there are scientific rules governing it. Using a computer algorithm we developed, we have been able to create maps of gene networks in which such interactions might occur due to sequence homology. One of these networks is now being examined in the nematode *Caenorhabditis elegans.* Two plasmid constructs have been created. The first targets four genes within the network for silencing through a common hairpin sequence found in each of the genes. The second construct targets just one these genes through sequence homology in an area outside of the hairpin. The two constructs, as well as a control construct, have been introduced into bacteria, induced to express the dsRNA and fed to the *C. elgans* to induce RNAi. Nematodes fed the first construct are phenotypically very similar to nematodes fed the second construct. Both exhibit impaired movement as compared *C. elegans* fed the control construct or no construct. This suggests that the pattern of silenced genes is similar between the two groups and that the silencing of the one gene with the second construct might have led to the silencing of the other three genes in manner that we could then begin to predict. We are currently examining RNA levels for all four genes with the *C. elegans* to determine if this is the case and will begin to examine the RNA levels of other genes within the network as well.

07 USE OF MOLECULAR MARKERS TO DETERMINE STRUCTURE OF COCOA TREE POPULATIONS IN TWO PERUVIAN VALLEYS

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The cocoa tree, *Theobroma cacao*, is native to the Amazon River region of South America, and diverse cocoa populations are thought to be in the river valleys of Peru. South American cocoa crops are increasingly threatened by witches' broom disease, and international collaborative projects are searching for methods of dealing with this problem. Although the germplasm repositories in Peru are regarded as a potential source of disease resistance, problems with identity of accessions have interfered with efficient use and conservation of this germplasm. We used DNA profiling based on microsatellite (SSR) loci, analyzed by capillary electrophoresis, to identify accessions collected from the Huallaga and Ucayali valleys of Peru. Genetic diversity and population structure were analyzed. Nearly 10% of the 105 accessions were synonymously mislabeled. The germplasm from the managed population in the Huallaga valley was clearly separated from the semi-natural population in the Ucayali valley. Ucayali germplasm had higher genetic diversity and significant spatial correlation was detected between genetic distance and geographical distance by Mantel tests.

08 ANALYSIS OF DETERGENT EXTRACTION METHODS AND 1D SDS-PAGE FOR ENRICHEMENT OF PLANT MEMBRANE PROTEINS AND SUBSEQUENT DETECTION BY MS/MS

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Although 2D-PAGE is a major technique used in the plant proteomics field, the lack of resolution for hydrophobic-membrane proteins, low abundant proteins, and basic proteins is a real obstacle to profiling whole plant proteomes with this separation method. On the other hand, MudPIT, a LC-based peptide separation technique coupled with mass spectrometry, is able to overcome some of the deficiencies of 2D-PAGE. Still MudPIT is not readily compatible with common detergents that are useful for extracting hydrophobic proteins from a sample. With this in mind, we have attempted to enrich for membrane proteins using various detergents and detergent clean-up methods that may be compatible with mass spectrometry. In comparison to our standard MudPIT methods, 1D SDS-PAGE separation followed by sequential MS/MS analysis of peptides eluted from the gel resolved several hundred more proteins. Alone, the 1D SDS-PAGE gel method is simple and robust, and is compatible with ion trap mass spectrometers coupled with an autosampler, HPLC pump, switching valve and microspray source. This method should be of interest to biologists who are looking beyond 2D-PAGE MS/MS to identify plant proteins but do not have MudPIT capabilities. We also show that the 1D SDS-PAGE separation process is compatible with detergents that are part of commercial membrane protein enrichment protocols, which are not compatible with routine MudPIT protocols. However, these commercial kits do not necessarily enrich for an overwhelming amount of membrane-spanning proteins as determined by ARAMEMNON, a public portal useful for identifying proteins with membrane spanning domains. Finally, we examined docecyl beta-maltoside (DBM), an acid-labile detergent that is compatible with mass spectrometry. DBM increased protein solubilization over our standard procedures and allowed us to detect more proteins. We conclude that combined DBM/MudPIT and 1D SDS-PAGE analyses are beneficial for sampling a large number of proteins. Several replicates of each analysis will help resolve a variety of proteins, which can result in increased coverage of membrane-spanning proteins by virtue of increased sampling. This project is supported by CRIS 1275-21220-214-00D and the FY05 Postdoctoral Research Associate Program.

09 PROTEIN PANORAMICS: PROBABILITY AND PARSIMONY-BASED SOFTWARE FOR ASSESSING PROTEINS ASSEMBLED FROM PEPTIDES INFERRED FROM MS/MS DATA

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Along with analyzing the quality of the peptide inferences from LC-MS/MS experiments, it is important is to reassemble the peptides into the proteins from which they were derived. This can be done by using a protein database as a scaffold. Since peptides can be assigned to different proteins due to protein sequence homology, there are often many proteins that contribute nearly identical peptides for the spectral identifications and the search results can be broadly interpreted to reflect that many proteins in the database were identified. However, there is a more realistic possibility that not all of the candidate proteins were present in the experimental sample. Thus to balance what is possible and what is logical, we have designed a software platform, PANORAMICS, that applies a rigorous probability model and assembles a parsimonious set of proteins from the deduced peptide sequences that best explains the observed data. PANORAMICS factors spectral inference scores, the number of peptides in the database with similar molecular weights, the charge state of the ions, the number of proteins sharing the same peptide sequence and length of peptide in relation to the size of search database and calculates a probability for a protein or group of proteins that share the same peptides. New concepts of peptide class and protein class are introduced in this software to help lower false positive rates and eliminate side-effects of distraction. Analysis of *Arabidopsis thaliana* MS/MS data by Mascot against the *A. thaliana* protein database (TAIR V. 6.0) and selection for peptides for which there is less than a 5% chance of the inferences being false-positive resulted in a non-redundant data set of 346 proteins. A search against a reverse *A. thaliana* protein database produced 39 non-redundant proteins, indicating a false positive rate of 11%. On the other hand, PANORAMICS assembled 265 non-redundant proteins with > 95% probability. The reverse database search produced 10 proteins at 95% level, indicating a reasonable false positive rate of 4%. PANORAMICS offers a unique feature of allowing the user to discard protein assemblies based on known experimental information, at which time probabilities can be recomputed. Novel data structures allow processing a 1 Gb Mascot .dat file in 40 seconds and the algorithm works equally well for both redundant and non-redundant databases.

10 ANALYSIS OF SPATIAL GENE EXPRESSION IN ARABIDOPSIS INFLORESCENCE BY MASSIVELY PARALLEL SIGNATURE SEQUENCING (MPSS)

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We have further elucidated the transcriptional complexities of organ localized expression in the model plant *Arabidopsis thaliana* by employing massively parallel signature sequencing (MPSS). Five libraries of sequence tags corresponding to RNA extracted from immature inflorescence tissues were constructed and matched to their respective loci in the annotated *Arabidopsis thaliana* genome. These signature libraries survey the floral transcriptome of wild type tissue as well as that of the floral homeotic mutants *apetala-1-10, apetala-3-6, agamous,* and *superman/apetala 1-10* in the Col-0 ecotype. With the aid of a dummy variable based system, comparative *in silico* analysis of these expression libraries permitted a genome wide dissection of organ specific expression as measured by MPSS. Transcripts expressed specifically in the petal, carpel, stamen, and those specific to the petal/stamen, or carpel/stamen were identified and quantified. The bulk of characterized transcripts were expressed in the gynoecium and androecium whereas fewer genes were localized in the perianth. This exemplifies the molecular intricacies associated with reproductive organs as opposed to vegetative floral tissue. Validation of the computational analysis among MPSS floral libraries was performed by comparison of previous expression data, *in situ* hybridization, and promoter-GUS fusions. This analysis has illustrated the accuracy of MPSS at assigning spatial floral expression and bolsters support for the application of MPSS expression library analysis (LIBAN) toward preliminary genome-wide functional analysis.

11 CHARACTERIZATION OF MAJOR ALLERGEN PROTEINS OF SOYBEAN BY PROTEOMIC TOOLS

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We investigated profiles of three major allergen proteins (Gly m Bd 60K, Gly m Bd 30K and Gly m Bd 28K) in sixteen different soybean genotypes that included four groups; wild, cultivated, modern (elite), and ancestor. Four genotypes were studied within each group. All allergen proteins were well separated by two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and characterized using mass spectrometry. Variation in the overall appearance and distribution patterns of allergen proteins were observed among the different genotypes. In wild genotypes the α-subunits of β-conglycinin separated into 6 or 7 protein spots whereas only 2-6 spots were evident in cultivated genotypes. Genotypes of modern and ancestor groups showed 2-5 protein spots. In contrast, glycinin G2 acidic polypeptide showed 4-5 spots in all genotypes. The G2 basic polypeptide showed 1-3 spots in wild, compared to 2 spots in cultivated, modern and ancestor soybean genotypes. One protein spot was detected for Gly m Bd 30K/P34 in wild compared to 1 or 2 spots in cultivated and 2 spots in other genotypes. Two protein spots were detected for Gly m Bd 28K in all genotypes except one modern genotype (PI 513382) that had one protein spot and no allergen spot was detected in one of the ancestor genotype (PI 548362). Major proteomic variation was observed between wild and cultivated soybean genotypes rather than among genotypes in the same group.

12 TRANSCRIPTIONAL RESPONSE IN APPLE TO FIRE BLIGHT DISEASE

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Fire blight, caused by the bacterium *Erwinia amylovora*, is a destructive disease of apple, pear, and other plants in the subfamily Maloideae of the Rosaceae. The goal of this study was to use a global analysis of gene expression to characterize the temporal response of apple to infection by *E. amylovora*. cDNA subtractive hybridization was used to compare the populations of mRNA in mock inoculated (buffer controls) and *E. amylovora* inoculated 'Gala' apple leaves at time intervals after challenge and to obtain EST clones. By subtracting cDNA synthesized from mRNAs expressed in one state (mock inoculated) from cDNAs derived from mRNAs expressed in another state (fire blight challenged) one can obtain sequences that are modulated when comparing the two mRNA populations side-by-side because sequences common to both populations are removed by hybridization. Gel electrophoresis of PCR-amplified subtracted cDNAs and unsubtracted controls indicate a greater quantity and size diversity in reverse subtracted samples (down-regulated ESTs) collected at 1h and 2h in comparison to forward subtracted samples (up-regulated) at 1h, 2h, 12h, pooled early samples (15 min, 1 h, 2h, 6h, 12h, 24h), and pooled later samples (48h, 72h), or in comparison to reverse subtracted samples (down-regulated) at 24h and 48h. PCR amplified subtracted cDNAs were cloned, sequenced, and identified by BLAST analysis.

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13 AN ASSESSMENT OF THE GENE FLOW OF SOLIDAGO SEMPERVIRENS ON THE EASTERN AND WESTERN SHORES OF THE CHESAPEAKE BAY

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This study used microsatellite data to compare genetic profiles among populations of goldenrod (*Solidago sempervirens* L.) growing on the eastern and western shores of the Chesapeake Bay. Goldenrods are insect-pollinated plants that are frequent in mesic habitats along the Chesapeake Bay and Atlantic coast of Maryland. We hypothesized that comparison of microsatellites among populations on the same side of the Bay with those on opposite sides of the bay will assist in identifying gene flow and the extent of population substructure along the bay. Preliminary data from one primer and three sites indicate some differences among populations. Primer 1B amplifies a DNA region that ranges in size from 156-210bp. This primer used on eight individuals from populations in St. Mary's, Calvert and Somerset counties isolated three alleles. Allele 1 (179bp) was common to all three populations. Alleles 2 (173bp) and 3 (188bp) were present in St. Mary's and Calvert Counties, respectively. The Somerset County population shared allele 1 with the other two populations. These results indicate the potential use of microsatellites to identify genetic exchange and population substructure in *S. sempervirens*.

14 THE RESEARCH OF MRNA TARGETING THROUGH POST-TRANSCRIPT RNAi

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Dr Rawn and his research students at Towson university have created a computer algorithm that predicts the pattern of silencing effects on certain genetic sequences cause by small interfering RNA (siRNA) that have targeted certain mRNAs. In Dr. Dwyer's lab we have used this algorithm on the genome of the organism ***C. elegans*** and targeted four "hairpin shaped" mRNA's to be "knock out" or silenced. The algorithm predicts which other genes will be silenced resulting in a domino-like effect from the 4 targeted mRNA's (2). In Dr. Dwyer's lab we are currently testing the genetic maps created by the to see if the predicted pattern of silencing can actually be produced in ***C. elegans***. The research has been broken down into two phases. Phase one of the research is to target the 4 chosen mRNA's from the algorithm, pqN80,Y37E3,F23C8.12 and Ankyrin, all of which carry the exact same genetic sequence in the hairpin region. In phase two, just one gene out the four, the Y37E3.13 gene, is targeted. The aim is to target the Y37E3.13 gene away from the hairpin and see if the pattern of silencing from phase one can be recreated. The hypothesis is that the double stranded RNA in the hairpin portion of each mRNA will allow the RNA interference machinery to recognize all of four of the targets. Currently in the lab, " Primer Express" Software 2.0 is being used to create primers and mRNA levels are being determined in c. elegans that have gone through phase one or phase two by "Real-time" PCR.

15 ISOLATION OF DEVELOPMENTALLY REGULATED GENES USING MICROARRAYS AND LASER CAPTURE MICRODISSECTION (LCM) OF GLYCINE MAX (SOYBEAN) SYNCYTIA FORMED BY THE PLANT PATHOGEN HETERODERA GLYCINES, (SOYBEAN CYST NEMATODE)

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The soybean cyst nematode (SCN), *Heterodera glycines*, is the major pest of soybean and causes an estimated one-half to one billion dollars in damage each year in the US. Chemical control for soybean is not normally used because economic and environmental costs are prohibitive. Therefore, agricultural practices, such as crop rotation and the use of resistant cultivars, are used to mitigate the damage of SCN. Our laboratory's research goal is to engineer transgenic soybean using multiple approaches to provide the farmer with high yielding soybean varieties with broad, durable resistance to SCN. One promising approach is the identify genes expressed at the nematode feeding site in roots of soybean during the resistance response and over-express them in susceptible soybean to improve resistance.

To identify genes expressed in soybean at the nematode feeding site, roots of soybean, *Glycine max* L. Merr., plants susceptible and resistant to the soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe, were inoculated with either SCN race 3, producing a resistant phenotype, or race 14, producing a susceptible phenotype. Feeding sites (syncytia) were collected using laser capture microdissection (LCM). RNA was extracted and used to make cDNA libraries and expressed sequence tags (ESTs) were produced and analyzed. The RNA was also used to study global gene expression using microarrays. We identified and isolated numerous genes involved in resistance and susceptibility to SCN invasion. Several of these genes may be useful in broadening resistance of soybean to SCN.

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