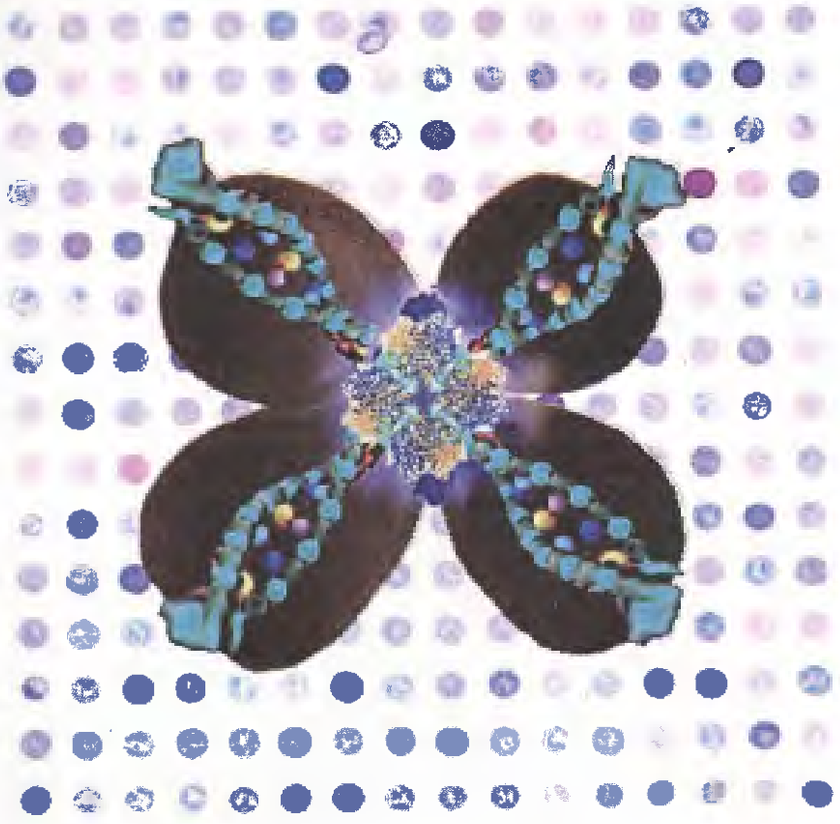
Mid Atlantic Plant Molecular Biology Society

22nd Annual Meeting



National Wildlife Visitors Center
Pautuxent Research Refuge

August 11-12, 2005

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COVER DESIGN, **Kelly Lagor**, George Washington University Dept. Biological Sciences

INTRODUCTION

On behalf of the Organizing Committees I would like to welcome you to the twenty-first meeting of the Mid-Atlantic Plant Molecular Biology Society, MAPMBS 2005. 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 & Leslie Wanner
Co-chairs

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2005 MAPMBS MEETING SCHEDULE

THURSDAY, AUGUST 11

9:00 AM Registration and Poster set-up

9:40 AM Introductions

Session I A - New Technologies in Gene Expression/Genetic Engineering
Moderator: Tara VanToai, USDA-ARS, Columbus, OH (TIGR, Rockville)

9:45 AM **Tim Bourett**, Dupont Crop Genetics Research and Development, Dupont Experimental Station, Wilmington, DE

DIGITAL IMAGING AND GENETIC ENGINEERING – A VERY POWERFUL COMBINATION FOR CELL RESEARCH

10:20 AM **Vincent Klink**, United States Department of Agriculture, Soybean Genomics and Improvement Laboratory, Bldg. 006, Beltsville, MD

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

10:40 AM **Break – Coffee, Exhibitors, Poster Set-up and Viewing**

11:10 AM **Paul Haynes**, Bio5 Institute and Department of Biochemistry, The University of Arizona, Tucson, AZ

FUNCTIONAL PROTEOMICS OF EXPRESSED ORPHAN PROTEINS IN RICE

11:45 AM **Pamela Green**, Delaware Biotechnology Institute, University of Delaware, Newark, DE

ELUCIDATING THE SMALL RNA COMPONENT OF THE TRANSCRIPTOME WITH MASSIVELY PARALLEL SIGNATURE SEQUENCING (MPSS)

12:05 PM Lunch – Exhibitors and Poster Viewing

Session IIA - Plant-Pathogen Interactions

Moderator: Margaret Jones, USDA-APHIS Biotechnology Regulatory Service, Riverdale MD

1:20 PM **C. Jacyn Baker**, Molecular Plant Pathology Lab, USDA-ARS, Beltsville, MD

REDOX MECHANISMS AND PLANT STRESS

1:50 PM **Christopher Dardick,** USDA-ARS Appalachian Fruit Research Station, Kearneysville, WV

PLANT AND ANIMAL PATHOGEN RECOGNITION RECEPTORS SIGNAL THROUGH NON-RD KINASES

2:10 PM **Pamela Ronald**, Department of Plant Pathology, University of California, Davis, CA

SIGNALING IN THE RICE XA21-MEDIATED DEFENSE RESPONSE

2:40 PM **Richard Sicher,** USDA-ARS Crop Systems & Global Change Lab, Beltsville, MD

EFFECTS OF ENHANCED ATMOSPHERIC CARBON DIOXIDE ON THE URUSHIOL CONTENT OF POISON IVY LEAVES

3:00 PM **Break – Exhibitors, Poster Viewing**

3:30 PM **Keynote Address**

Gregory B. Martin, Boyce Thompson Institute for Plant Research and Department of Plant Pathology, Cornell University, Ithaca, New York

MOLECULAR MIMICRY IN PLANT- PATHOGEN INTERACTIONS

Introduction: John Hammond, USDA-ARS Floral and Nursery Plants Research Unit, US National Arboretum, Beltsville, MD

4:30 PM **Close of day**

FRIDAY, AUGUST 12

Session IB – New Technologies in Genomics

Moderator: June Kwak, Department of Cell Biology & Molecular Genetics, University of Maryland, College Park MD

9:30 AM **Pablo Rabinowicz**, The Institute for Genomic Research, Rockville, MD

USING GENE ENRICHMENT TECHNIQUES TO SEQUENCE LARGE PLANT GENOMES

10:00 AM **Bret Cooper,** USDA-ARS Soybean Genomics and Improvement Laboratory, Beltsville, MD

SHOTGUN IDENTIFICATION OF PROTEINS FROM RUST SPORES

10:20 AM **Break – Exhibitors and Poster Viewing, Coffee**

10: 50 AM **Bradley J. Till**, Fred Hutchinson Cancer Research Center, 1100 Fairview Avenue North, Seattle, Washington

HIGH-THROUGHPUT TILLING FOR FUNCTIONAL GENOMICS

11:20 AM **Nadim Alkharouf,** Soybean Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD

IDENTIFICATION OF SOYBEAN CYST NEMATODE LETHAL GENES USING COMPUTATIONAL METHODS

11:40 AM **Business Meeting**

12:00 PM **LUNCH: Exhibitors and Poster Viewing**

Session IIB - Plant-Pathogen Interactions

Moderator: Catherine A. Preston, USDA-APHIS Biotechnology Regulatory Service, Riverdale MD

1:15 PM **David M. Francis**, Department of Horticulture and Crop Science, The Ohio State University, OARDC, Wooster, OH

INSIGHTS INTO QUANTITATIVE DISEASE RESISTANCE THROUGH PROTEOME ANALYSIS OF THE TOMATO-CLAVIBACTER INTERACTION

1:45 PM **Nigel Gapper,** Produce Quality & Safety Lab, USDA-ARS, Beltsville, MD

INHIBITION OF ETHYLENE-INDUCED ALPHA-FARNESENE SYNTHASE GENE EXPRESSION IN D'ANJOU PEARS WITH 1-MCP DELAYS ALPHA-FARNESENE SYNTHESIS AND OXIDATION AND PREVENTS SUPERFICIAL SCALD

2:05 PM **Steve Whitham**, Iowa State University, Ames, IA

REGULATION OF HOST GENE EXPRESSION IN RESPONSE TO VIRAL INFECTION

2:35 PM **Closing Remarks**

Take Down Posters

2005 MAPMBS Poster Session

Poster Page

Isolation and analysis of a cytoplasmic dynein from the soybean pathogen Heterodera glycines

7 39 **Savithiry Natarajan*, Perry Cregan[a], Chenping Xu[b], Thomas J. Caperna[c], Wesley M. Garrett[c] and Joseph Sullivan[b]** * [a]USDA-ARS, Soybean Genomics and Improvement Laboratory, PSI, Beltsville, MD 20705; [b]University of Maryland, Department of Natural Resource Sciences and Landscape Architecture, College Park, MD, 20742; [c]USDA-ARS, Growth Biology, Biotechnology and Germplasm, and Bovine Functional Genomics Laboratories, Beltsville, MD

Proteomic Analysis of β-Gonglycinin and Glycinin in Different Soybean Genotypes

8 40 **Neerav D. Padliya[1], Bret Cooper[1] and Troy D. Wood[2]**, [1]Soybean Genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD; [2]Department of Chemistry, University at Buffalo, Buffalo, NY

Negative-Ion Mode Nanoelectrospray Tandem Mass Spectrometry: Potential to Improve the Detection of Pathogen Proteins

9 41 **Dhananjay Naik, Anik L. Dhanaraj, and Lisa J. Rowland*** USDA/ARS, Fruit Laboratory, Bldg. 010A, BARC-West, Beltsville, MD 20705

Identification of cold-responsive genes in blueberry (Vaccinium corymbosum L.) using a subtractive hybridization approach

10 42 **Banu Saritas-Yildirim and Gary Coleman**, Department of Natural Resource Sciences and Landscape Architecture, University of Maryland, College Park, MD

RUB1 Conjugase and Vegetative Bud Development

11 43 **C. Srinivasan[1], Zongrang Liu[1], Ralph Scorza[1], Michael Glenn[1], Jan B.M. Custers[2] and Kim Boutilier[2]**, [1]United States Department of Agriculture- Agricultural Research Service, Appalachian Fruit Research Station, 2217 Wiltshire Road, Kearneysville, West Virginia 25430, USA; [2]Plant Research International, P.O. Box 16, 6700 AA Wageningen, The Netherlands

Morphological and physiological alterations in transgenic tobacco (Nicotiana tabacum L.) due to the ectopic expression of Arabidopsis thaliana and Brassica napus baby boom genes

12 44 **Veronica Martins, Vincent Klink, Nadim Alkharouf, Margaret MacDonald, and Benjamin Matthews**, USDA-ARS, Soybean Genomics

and Improvement Laboratory, 10300 Baltimore Ave., Bldg. 006, Beltsville, MD

Isolation and characterization of the soybean cyst nematode (Heterodera glycines) homolog of Caenorhabditis elegans unc-97 gene

13 45 **Anjana R. Vatsan, Andrey Kochergin, Sergei Resenchuk, Kim Pruitt, Tatiana Tatutsova**, National Center for Biotechnology Information, National Library of Medicine, National Institutes of Heal

Plant projects in the Entrez Genome Project database

14 46 **Vijayakala Vydeeswaran and David C Straney**, Molecular and Cell Biology Program, Dept of Cell Biology and Molecular Genetics, HJ Patterson, University of Maryland, College Park, MD

Fungal response to plant defense compounds: A two-hybrid approach to dissect the signal transduction cascade

15 47 **P. Xue and M. L. Tucker**, USDA-ARS, Soybean Genomics and Improvement Laboratory, Bldg.006, BARC-West, 10300 Baltimore Ave., Beltsville, MD

Effect of ethylene and its inhibitors on the number of soybean cyst nematodes in soybean cyst nematode-infected soybean roots

16 48 **Chenping Xu[a], Wesley M. Garrett[b], Joseph Sullivan[a], Thomas J. Caperna[c] and Savithiry Natarajan[d]** [a]Department of Natural Resource Sciences and Landscape Architecture, University of Maryland, College Park, MD ; [b]Biotechnology and Germplasm Laboratory, [c]Growth Biology Laboratory, [d]Soybean Genomics and Improvement Laboratory, PSI, USDA-ARS, Beltsville, MD 20705, USA

Separation and Identification of Soybean Leaf Proteins by 2D-PAGE and Mass Spectrometry

DIGITAL IMAGING AND GENETIC ENGINEERING – A VERY POWERFUL COMBINATION FOR CELL RESEARCH

Tim Bourett, Richard Howard, Keith Duncan and Nancy Rizzo

Dupont Crop Genetics Research and Development, Dupont Experimental Station, Wilmington, DE

timothy.m.bourett@cgr.dupont.com

The exploitation of genetically encoded fluorescent proteins and recent advancements in fluorescence-based digital instrumentation have been combined to generate novel findings in all areas of biology. This approach has allowed researchers to (1) tag organisms, (2) localize specific proteins, (3) assess the efficiency of cell- and tissue-specific promoters using promoter-FP fusion proteins, (4) conduct targeting/compartmentalization analyses and document organelle dynamics, (5) track and quantify specific protein populations, e.g. using photoactivatible fluorescent proteins or applying photobleaching methodologies, (6) assess protein-protein interactions, e.g. using fluorescence resonance energy transfer (FRET) or bimolecular fluorescence complementation, and (7) investigate biological events and signals using biosensors. A variety of fluorescent proteins is now available. The non-invasive three-dimensional quality, high contrast and spatial resolution afforded by confocal and multiphoton microscopy are extremely useful for imaging fluorescent proteins and has pushed the limits of photon-based microscopy. The ability to observe molecular interactions in real time provides exciting new opportunities for understanding biological processes. Since proper functioning requires that molecules be at the appropriate place, and time, the advantages of time-resolved live-cell imaging cannot be overemphasized. Confocal microscopes employ a pinhole aperture to reject out-of-focus and/or scattered light to increase the signal-to-noise ratio and create an optical slice representing a sharp thin plane within the sample. Multiphoton microscopy extends the potential for optical sectioning deeper into samples with a pulsed near infra-red laser light source that can be less injurious to living cells. Confocal and multiphoton microscopy allows for monitoring of developmental and environment-induced changes over time and in three dimensions. Such spatiotemporal resolution is especially important when studying ephemeral specimens, or targets, such as host-pathogen interactions. Data acquisition can be enhanced significantly using a computer controlled microscope stage. Spectral confocal imaging represents yet another recent advance that allows for separation of fluorescent signals with grossly overlapping emission spectra, and increases the number of fluorescent reporter molecules that can be imaged simultaneously. Since many fluorescent proteins have broad multi-photon cross-sections (i.e., excitation spectra) making it difficult to excite them independently spectral confocal imaging may be important especially when conducting multi-label experiments, as a strategy for eliminating cross talk. One disadvantage of laser scanning instruments has been the relative slow speed of acquisition. Spinning disc microscopes have been used to record rapid cellular events but at a cost reduced confocality, less flexibility in lens choice, and incompatibility with more sophisticated types of analysis such as photobleaching-based methodologies. Exciting recent developments in rapid acquisition confocal systems show much promise in eliminating the compromise between speed of acquisition and high image quality.

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

Vincent Paul Klink[1], Margaret MacDonald[1], Nadim Alkharouf[1, 2], and Benjamin Matthews[1]

[1]United States Department of Agriculture, Soybean Genomics and Improvement Laboratory, Bldg. 006, Beltsville, MD 20705; [2] Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742-2350
KlinkV@ba.ars.usda.gov

The soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe is the major pest of soybean and causes an estimated $750 million in losses each year in the U.S. No soybean cultivar has been identified with resistance to all races of SCN. We are developing methods to identify genes specifically expressed at the feeding site developed by SCN in susceptible and resistant roots of soybean. Some of the genes identified may be useful in broadening resistance of soybean to SCN through genetic engineering. To this end, roots of soybean, *Glycine max* cv. Kent L. Merr., plants susceptible to SCN, were inoculated and allowed to develop feeding sites (syncytia). Syncytial cells were isolated and collected using laser LCM. RNA was extracted from the isolated syncytia and used to make a cDNA library. Expressed sequence tags (ESTs) were produced and analyzed. The cDNAs encoding unique α-tubulin (GmTubA1), β-tubulin (GmTubB4), an aquaporin (GmPIP2,2) and a resistance gene were identified by EST analyses. Immunolocalization and image analyses within and around the syncytium indicated increased levels of α-tubulin within the syncytium. However, α-tubulin labeling appeared diffuse or clumped. RT-PCR results indicated enhanced expression of GmTubA1, GmTubB4, GmPIP2,2 and several other genes including a pathogen resistance gene in syncytium-enriched samples as compared to samples extracted from whole roots. The isolation of RNA from syncytia has also provided material suitable for microarray analysis of syncytia. The genes identified in syncytia from susceptible roots will be compared with those expressed in syncytia of resistant roots to identify genes that may be candidates for over-expression in transgenic soybean to broaden soybean resistance to SCN.

FUNCTIONAL PROTEOMICS OF EXPRESSED ORPHAN PROTEINS IN RICE

Paul A. Haynes

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Bio5 Institute and Department of Biochemistry, The University of Arizona, Tucson, AZ, 85721

Our goal is to perform functional proteomic characterization of expressed orphan proteins in *Oryza sativa* (rice), with the objective of creating a resource for the plant biology research community. We will first undertake an exhaustive proteomic survey of leaf, root, seed and callus tissue from rice, and extract from this data a subset of expressed proteins consisting of all those that are identified, yet have no known function or homology to known proteins. These orphan proteins will then be expressed as TAP-tagged transformants in rice callus tissue, and we will use mass spectrometric techniques to identify the binding partners of each orphan in immunoprecipitation pull-down experiments. This data will be used to infer an initial protein function on the basis of protein-protein interactions identified. All of this data will be integrated with existing rice genome database resources, and made publicly available. We expect this project to serve as a starting point for many future research programs based on further detailed characterization of the expressed orphan proteins for which we will provide an initial functional annotation.

We have begun collecting data for our proteomics analysis, specifically from SDS-PAGE – nanoLC-MS/MS and 2D-nanoLC-MS/MSof rice leaf, root, seed and callus. Protein extracts were prepared by grinding and TCA/acetone protein precipitation. MS/MS spectra were searched against a combined plant protein database including copies of the translated rice and Arabidopsis genome sequences. Applying high stringency results filtering, the combined non-redundant total of proteins identified in our experiments so far is just over 2000 distinct proteins, which includes a small number of expressed orphan proteins. We are currently in the process of PCR amplification and vector construction for TAP tagging the first group of expressed orphan proteins identified, and initial transformants are being generated.

ELUCIDATING THE SMALL RNA COMPONENT OF THE TRANSCRIPTOME WITH MASSIVELY PARALLEL SIGNATURE SEQUENCING (MPSS)

Cheng Lu[1], Shivakundan Singh Tej[1], Shujun Luo[2], Christian D. Haudenschild[2], Blake C. Meyers[1], and Pamela J. Green[1]

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[2] Solexa, Inc., 25861 Industrial Blvd., Hayward, California 94545
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Small RNAs play important regulatory roles in most eukaryotes but only a small proportion of these molecules have been identified. We have developed a novel method for the identification and measurement of small RNAs that provides a substantial advance over existing methods for the analysis of these RNA molecules. This method is based on massively parallel signature sequencing ("MPSS") and can sequence hundreds of thousands of molecules in parallel. From two libraries representing Arabidopsis flower and seedlings, we identified more than 75,000 different small RNA sequences. We are combining experimental and bioinformatics approaches to gain insight about the regulation, genomic distribution and roles of these molecules This powerful genome-wide approach extends miRNA prediction capabilities and is applicable to diverse organisms. Funded by the National Science Foundation.

REDOX MECHANISMS AND PLANT STRESS

C. Jacyn Baker

Molecular Plant Pathology Lab, USDA-ARS, Beltsville, MD

Oxidative metabolism and the reactive oxygen species (ROS) generated by it, impact numerous facets of normal life in both plants and animals and have an even greater impact during times of disease and environmental stress. Oxidative metabolism as a field of study is not well defined but can be viewed as a collection of reactions in which electrons are either lost or gained (including redox reactions, light reactions, electron transport, and reactive oxygen reactions). Although these reactions are not necessarily part of the same biochemical pathway, they influence each other by their direct effect on the redox environment (which governs how readily electrons are exchanged). New studies continually reveal the comprehensive connection of oxidative metabolism to nearly all aspects of life and metabolism. Changes in cellular redox status have been shown, to co-regulate signal transduction, transcription factors, kinase and phosphatase activation, protein synthesis, enzyme activation and the cell cycle/division.

The potential to exploit oxidative metabolism through biotechnology to improve disease resistance in plants and animals is intriguing. However in doing so, there are two very different aspects to "oxidative stress" that must be considered. One is the direct oxidation of surrounding molecules by ROS, which can be countered to various degrees by antioxidative processes. The second is the oxidative signaling mechanisms that are triggered by changes in redox status and which lead to various regulatory responses by the host.

Initial attempts to manipulate oxidative metabolism to improve plant resistance to either environmental or pathogenic stress have followed two narrow and almost juxtaposed strategies and met with limited success. Chronic low level increases in ROS have been associated with many environmental stresses. Therefore, bioengineering to increase resistance to environmental stress has focused on increasing the plant antioxidant systems. An almost opposing strategy was used to improve disease resistance, where increased albeit transient levels of ROS have been associated with resistance. Transgenic plants were developed with increased levels of endogenous ROS, specifically H_2O_2, to increase resistance to disease. The success of these studies was generally limited to the narrow criteria of the particular study. In some cases, resistance to stress paradoxically was decreased. These opposing strategies for developing resistant plants point out the need for a broader approach and understanding of oxidative metabolism.

PLANT AND ANIMAL PATHOGEN RECOGNITION RECEPTORS SIGNAL THROUGH NON-RD KINASES

Christopher Dardick[1] and Pamela Ronald[2]

[1]USDA-ARS Appalachian Fruit Research Station, Kearneysville, WV. [2]University of California, Davis, CA.

cdardick@afrs.ars.usda.gov

Plants and animals mediate early steps of the innate immune response through pathogen recognition receptors (PRRs). PRRs commonly associate with or contain members of a monophyletic group of kinases that include *Drosophila* Pelle, human IRAK, rice XA21 and *Arabidopsis* FLS2. In addition to their phylogenetic similarities, we have found that these kinases also fall into a small functional class of kinases termed non-RD. Non-RD kinases, unlike the more common RD kinases, do not typically auto-phosphorylate a regulatory region called the activation loop. This suggests that the regulatory mechanisms of kinases mediating early steps of innate immunity signaling are conserved in plants and animals. We surveyed the human, fly, worm, *Arabidopsis* and rice kinomes (3,122 kinases) and found that despite the small number of non-RD kinases in these genomes (9-29%), nearly all kinases associated with PRRs fall into the non-RD class. Furthermore, plant non-RD receptor kinases show evolutionary expansion patterns consistent with that of NBS-LRRs, supporting a role for these kinases in pathogen recognition. These data indicate that kinases associated with PRRs can largely be predicted by the lack of a single conserved residue.

SIGNALING IN THE RICE XA21-MEDIATED DEFENSE RESPONSE

Pamela. C. Ronald

Department of Plant Pathology, University of California, Davis

Components of innate immune systems in both plants and animals share many conserved features. Most notably, they sense the presence of pathogen-associated molecules (PAMs), which represent conserved molecular structures, and avirulence (Avr) factors that are strain specific molecules produced by phytopathogens. Given the importance of these proteins in innate immune recognition and host defense, there is great interest in identifying the PAMs that they detect as well as elucidating the downstream signaling cascades that they induce.

The rice Xa21 receptor kinase mediates recognition of *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strains expressing AvrXa21 activity. We have identified eight *Xoo* genes, falling into three classes, which are required for AvrXa21 activity. These genes encode components of a Type I secretion system (*raxA*, *raxB* and *raxC*), enzymes involved in sulfur metabolism (*raxST*, *raxP* and *raxQ*) and components of a two-component regulatory system (*raxH* and *raxR*). *raxST*, *raxA* and *raxB* are part of an operon (*raxSTAB*) that has been shown to be regulated by the RaxH/RaxR system. Based on our results, we hypothesize that upon sensing of the plant environment, the AvrXa21 molecule is sulfated and then secreted by the RaxABC Type I secretion system making it available for race specific interactions with the rice receptor kinase XA21.

Upon AvrXa21/Xa21 interaction, the XA21-mediated defense response is activated. Components of this response include Xb10, encoding a putative transcriptional regulator; Xb15 encoding a PP2c phosphatase-like protein and NRR, an NPR1 and NH1 interacting protein.

EFFECTS OF ENHANCED ATMOSPHERIC CARBON DIOXIDE ON THE URUSHIOL CONTENT OF POISON IVY LEAVES

J. Mohan, K. George, L. Ziska & R. Sicher

Crop Systems & Global Change Lab., Beltsville Agric. Res. Ctr., Beltsville, MD 20705

Over 300,000 cases of contact dermatitis from poison ivy [*Toxicodendron radicans*] are reported in the Eastern United States each year. Compounds in poison ivy that react with the human immune system to produce skin rash are known as urushiols, a group of closely related 3-n-pentadecyl catechols. Poison ivy leaves were sampled monthly from May to September of 2004, using plants from the Duke University Free Air CO_2 Enrichment site. Three 707 m^2 plots each, supporting loblolly pine [*Pinus taeda* L.] as the dominant tree species, were maintained at ambient (37 Pa) and elevated (37 + 20 Pa) carbon dioxide. Poison ivy leaves were extracted with 95% ethanol, the homogenates were partitioned and the organic fraction was taken to dryness. Samples were derivatized by silynation and analyzed by GC coupled to a mass selective detector. Up to five individual urushiol congeners [the saturated form (m/e 464), two forms of the monoene (462), the diene (m/e 460) and the triene (m/e 458)] were detected in poison ivy leaf extracts. Quantitation was based on a partially purified urushiol standard from poison oak. Growth at elevated compared to ambient CO_2 increased photosynthetic rates (77%), growth rates (62%) and the population biomass (31%) of poison ivy. Also, the relative fraction of the saturated urushiol congener decreased from 0.10 to 0.06 and that of the triene increased from 0.17 to 0.42. Significantly, the bioactivity of poison ivy urushiols increases with the degree of unsaturation. The above results indicated that CO_2 enrichment produces larger more vigorous plants, greater population biomass and increases the allergenicity of poison ivy.

MOLECULAR MIMICRY IN PLANT-PATHOGEN INTERACTIONS

Gregory B. Martin, Robert B. Abramovitch, Jeffery C. Anderson, Jonathan R. Cohn, Nai-Chun Lin

Boyce Thompson Institute for Plant Research and Department of Plant Pathology, Cornell University, Ithaca, New York, USA gbm7@cornell.edu

We study the interaction between tomato leaves and Pseudomonas syringae pv. tomato as a model system to investigate the molecular basis of bacterial pathogenesis and host responses to bacterial attack. Recently, we have focused on two key Pseudomonas proteins, AvrPto and AvrPtoB, which are secreted by the bacterial type III secretion system into host cells where they promote disease susceptibility (1, 2). Our current efforts are directed at understanding the molecular basis of the virulence activity of these bacterial proteins. By using two-dimensional gel electrophoresis, in vivo 32P-labeling, and mass spectrometry we found that, when expressed in plant leaves, AvrPto is phosphorylated on serines in its C terminus. Phosphorylation of these sites is due to a kinase activity (termed Avk) that is present in tomato, tobacco, and Arabidopsis leaf extracts. Substitution of alanines for the phosphorylated serines decreases the ability of AvrPto to enhance disease symptoms and promote growth of *P.s.* pv. *tomato* in leaves. We hypothesize that AvrPto mimics a substrate of a conserved plant kinase and that phosphorylation is necessary for AvrPto to promote full bacterial virulence in plants.

Related to AvrPtoB, we found that this protein has a modular structure with the N- and C-terminal regions having distinct functions. The C-terminal region (CTR) acts as a virulence factor to broadly suppress programmed cell death (PCD) in plants and several lines of evidence suggest the CTR targets a conserved eukaryotic process (4). A yeast two-hybrid screen identified tomato ubiquitin as interacting with the CTR. Ubiquitin (Ub) is present in all eukaryotes and Ub-associated processes include protein turnover, signal transduction and disease resistance. Additional *in vitro* and *in vivo* experiments indicate that AvrPtoB is ubiquitinated. We identified a domain in the CTR that is required for the AvrPtoB-Ub interaction and for suppression of PCD. When delivered by *P. s.* pv. *tomato,* AvrPtoB mutants that no longer interact with Ub elicit plant immunity in otherwise susceptible tomato plants, revealing that AvrPtoB-Ub interactions are required for both suppression of PCD and for *Pseudomonas* pathogenicity. In summary, our observations of both AvrPto and AvrPtoB indicate that these proteins have evolved to mimic plant proteins in order to promote bacterial virulence.

1. Pedley and Martin (2003). Ann. Rev. Phytopathology 41:215.
2. Abramovitch, R., et al., (2003). *EMBO J* 22:60
3. Lin, N., et al., (2005). *Mol Plant-Microbe Interact* 18:43
4. Kim, Y., et al., (2002). *Cell* 109:589
5. Abramovitch, R., et al., (2004). *Curr Opin Plant Biol* 7:356

USING GENE ENRICHMENT TECHNIQUES TO SEQUENCE LARGE PLANT GENOMES

Pablo Rabinowicz[1], Agnes Chan[1], Geo Pertea[1], Li Zheng[1], Foo Cheung[1], Dan Lee[1], Brad Barbazuk[2], Karel Schubert[2], Roger Beachy[2], Phillip SanMiguel[3], Jianxin Ma[4], Ana Clara Pontaroli[4], W. Richard McCombie[5], Rob Martienssen[5], Jeffrey Bennetzen[4], Joseph A. Bedell[6], Robert W. Citek[6], Arief Budiman[6], Andy Nunberg, Nathan Lakey[6] and John Quackenbush[1]

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Maize is an excellent plant model system and an important crop. Therefore, the decision has been made to provide funding to sequence its 2.5 Gbp genome. Prior to this decision, gene-enrichment sequencing techniques were tested in maize in an attempt to identify new, more efficient technologies to capture the most relevant information from large and repetitive genomes. Techniques such as methylation filtration and high Cot sequencing proved successful in maize, which opens the opportunity to approach other even larger plant genomes for sequencing. Analysis of the maize gene-enriched sequences and pilot studies in other plant genomes will be discussed.

SHOTGUN IDENTIFICATION OF PROTEINS FROM RUST SPORES

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We are interested in learning more about the proteome of *Uromyces appendiculatus*, the causal agent of common bean rust. The knowledge of the proteins that form uredospores, germlings, appressoria, and haustoria may be used to define host-pathogen interactions or serve as targets for chemical inhibition of the fungus. We have used liquid chromatography tandem mass spectrometry (LC-MS/MS) in a high-throughput fashion to identify ~400 proteins from uredospores. An abundance of heat-shock proteins and translation elongation factors portends a spore's ability to survive stresses and jump-start protein production when germination is initiated.

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HIGH-THROUGHPUT TILLING FOR FUNCTIONAL GENOMICS

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The Seattle TILLING Project (STP, http://tilling.fhcrc.org:9366/) has developed high throughput methods for the discovery of induced point mutations (TILLING, Targeting Local Lesions IN Genomes) and for the discovery and genotyping of natural nucleotide polymorphisms (Ecotilling). Both technologies utilize PCR and gene specific primers to amplify a target region of the genome. Following a denaturation and reannealing step, mismatches are cleaved with a single-strand specific nuclease and the resulting products are visualized using denaturing polyacrylamide gel electrophoresis. Because the methods employed are general, TILLING and Ecotilling can be applied to many organisms. The main work of our facility can be grouped into four categories. First, we operate public services where researchers can request an allelic series of induced point mutations in their favorite target gene. For plants, we offer services for Arabidopsis (Arabidopsis TILLING Project, ATP) and Maize (Maize TILLING Project, MTP). Second, we perform pilot screens on a variety of plant species to evaluate mutation frequency and determine if the population is suitable for TILLING. Third, we disseminate the TILLING and Ecotilling technologies to other groups through workshops we hold at our facility. Finally, we seek to improve the TILLING and Ecotilling technologies. Recent advancements include moving from a 96 to a 384 platform, discovering nucleases in the S1 family that can cleave single nucleotide mismatches, and developing software to automate lane calling and facilitate mutation discovery.

IDENTIFICATION OF SOYBEAN CYST NEMATODE LETHAL GENES USING COMPUTATIONAL METHODS

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Soybean cyst nematode (SCN; *Heterodera glycines*) is a devastating pest of soybean causing one billion dollars in losses to the US economy per year and over ten billion worldwide. The genome sequence of SCN is not well characterized nor fully sequenced. On the other hand *Caenorhabditis elegans* genome is fully sequenced, well characterized and a number of lethal genes in *C. elegans* have been identified through experimental methods. *C. elegans* is a free living nematode while SCN is a sedentary root endoparasite, but both worms are homologous to each other in structure. We downloaded all the known *C. elegans* genes into a database and used it to blast approximately 1300 SCN known genes against it. We identified a number of SCN lethal genes through this simple yet effective method that have high homology to *C. elegans* lethal genes. These candidate genes will be used as RNAi targets to neutralize SCN and limit it's infection of soybean. An automated pipeline was developed for this purpose and is available upon request.

INSIGHTS INTO QUANTITATIVE DISEASE RESISTANCE THROUGH PROTEOME ANALYSIS OF THE TOMATO-*CLAVIBACTER* INTERACTION

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Bacterial Canker of tomato is caused by the Gram-positive bacterium *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*). The disease is potentially devastating due to systemic spread through the xylem. Partial resistance to *Cmm* in *Lycopersicon hirsutum* LA407 is controlled by two quantitative trait loci (QTL), *Rcm2.0* and *Rcm5.1*. These two loci interact to explain 60-70% of the phenotypic variation for resistance. We compared lines containing *Rcm2.0*, *Rcm5.1*, and a susceptible control using 2-dimensional gel electrophoresis. Patterns of protein expression in leaf and stem tissue suggest that the QTL mediate resistance through separate but complimentary mechanisms. Over 55 proteins were subjected to tandem mass spectrometry. Database queries with resulting spectra and interpretation of spectra to deduce peptide amino acid sequences were used to identify tomato genes or ESTs for 49 proteins. Pathogenesis-related proteins, enzymes mediating JA response, and enzymes capable of generating oxidants are differentially expressed in resistant and susceptible tomato lines following *Cmm* infection. Three superoxide dismutase enzymes and two peroxidases were differentially regulated between genotypes and patterns of hydrogen peroxide accumulation were genotype and tissue specific, indicating a role for oxidative stress in response to *Cmm*. Insights from these studies suggest possible mechanisms of virulence in *Cmm*, have application in germplasm screening for resistance in diverse germplasm, provide potential strategies for resistance breeding, and suggest that quantitative resistance may result from the additive affects of loci that confer resistance through distinct but complimentary mechanisms.

INHIBITION OF ETHYLENE-INDUCED ALPHA-FARNESENE SYNTHASE GENE EXPRESSION IN d'ANJOU PEARS WITH 1-MCP DELAYS ALPHA-FARNESENE SYNTHESIS AND OXIDATION AND PREVENTS SUPERFICIAL SCALD

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Several commercial cultivars of apple and pear fruit are prone to superficial scald, a storage disorder that appears as browning or blackening of the skin, which results from necrosis of the hypodermal cortical tissue. Tissue damage is thought to be induced or exacerbated by conjugated trienol (CT) oxidation products of the sesquiterpene alpha-farnesene, which accumulate in the fruit skin during cold storage. In apple fruit, ethylene is known to play a key role in development of scald. Pre-storage treatment with 1-methylcyclopropene (1-MCP), a blocker of ethylene perception, inhibits alpha-farnesene production and CT accumulation, and largely prevents scald development after 4-6 months of storage at 0-1°C. A marked increase in expression of AFS1, the gene encoding alpha-farnesene synthase (AFS), precedes the rapid accumulation of alpha-farnesene in cold-stored apples, and 1-MCP treatment blocks the up-regulation of AFS1 gene expression. AFS converts farnesyl diphosphate to alpha-farnesene, the last step in synthesis of this sesquiterpene via the mevalonic acid pathway, and AFS1 appears to be the only gene in the pathway that is up-regulated by ethylene in the first weeks of storage. The present study was conducted to determine if the same mechanism governs alpha-farnesene production in scald-susceptible d'Anjou pear. A gene encoding AFS (Pc*AFS1*) was cloned using RT-PCR with primers based on apple AFS1 and RNA from peel tissue of pears stored for 4-8 weeks. Control and 1-MCP-treated (0.3 ppm; 24 h) pears were stored for up to 6 months at –1°C in air. An increase in Pc*AFS1* expression occurred in control fruit over the first 3 months, but was greatly attenuated in 1-MCP-treated fruit. Alpha-farnesene and CTs in peel tissue extracts were quantified by HPLC-UV and scald incidence was scored at one-month intervals. Maximum levels of alpha-farnesene and CTs in control fruit occurred at 3 and 4 months, respectively, and scald incidence was 100% after 3 months. Alpha-farnesene and CT levels at these time points were, respectively, 9- and 19-fold lower in 1-MCP-treated fruit, which had no scald.

REGULATION OF HOST GENE EXPRESSION IN RESPONSE TO VIRAL INFECTION

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In *Arabidopsis thaliana* diverse viruses elicit both common and unique changes in host gene expression. About one-third of the genes induced in common are associated with defense and stress responses. Mutations in signaling pathways that normally control defense responses were tested to determine mechanisms involved in regulating gene expression in compatible host-virus interactions. Defense mutants or wild type plants were infected with *Cucumber mosaic virus* (CMV) or *Oilseed rape mosaic virus* (ORMV), and gene expression was interrogated using high-throughput fiber-optic bead arrays consisting of 388 *A. thaliana* genes. The expression of most defense-related genes was induced by a salicylic acid (SA)-dependent, *NPR1*-independent signaling pathway. Subsequent analyses demonstrated that *SID2* and *EDS1* were also required for the expression of these genes, and thus confirmed that SA and genes upstream of SA mediate host responses to compatible viruses. Members of the major families of heat shock proteins (HSPs) are also induced in plants in response to viral infections. The HSP70 and HSP100 families consist of 14 and 8 members, respectively, in *A. thaliana,* and each was included on the fiber optic bead array in order to obtain a comprehensive view of how their expression was affected by viral infection. Specific *HSP100* and *HSP70* genes were significantly up-regulated during viral infections, and their induction did not require the defense-related signaling pathways involving SA, jasmonic acid, and ethylene. HSP101 was the only member of the HSP100 family that was induced, and its induction was observed in response to ORMV, but not CMV, at both the mRNA and protein levels. The accumulation of *HSP101* and *HSP70* mRNA transcripts increased in response to inducible expression of the ORMV coat protein, demonstrating that it is an effective inducer of these heat shock genes.

CHARACTERIZATION AND SUBSTRATE INDUCED TRANSCRIPTIONAL REGULATION OF THE PENTAFUNCTIONAL AROM GENE OF RHIZOCTONIA SOLANI

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The shikimate pathway *arom* gene is involved in the aromatic amino acid production and as a virulence regulatory element in the basidiomycete *Rhizoctonia solani*. The complete *arom* gene and its transcript from *R. solani,* anastomosis group 3 have been characterized. The gene consists of 5,323 base pairs including five introns. The upstream sequence has a GC box, and two GTATTAGA repeats. The largest isolated *arom* transcript is 5,108 nucleotides long. It contains an open reading frame of 4857 bases, coding for a putative 1618-residue pentafunctional AROM protein. A consensus sequence for initiation of translation (GCGCCATGG) is present between +127 and +135 bases. The 5'-end of the transcript includes two nucleotides (UA) that are not found in the genomic sequence. Size and sequence heterogeneity were observed at both 5'- and 3'-ends of the mRNA. Northern blot and suppression subtractive hybridization analyses showed that presence of a low amount of quinate, inducer of the quinate pathway, resulted in increased levels of *arom* mRNA, consistent with the compensation effect observed in ascomycetes. Phylogenetic analyses of the putative *R. solani* AROM protein with sixteen AROM proteins belonging to three groups of ascomycetes and two AROM proteins from other basidiomycetes correlated with the widely accepted fungal systematics schemes.

THE ABI3 INTERACTING PROTEIN 6 (AIP6) GENE OF ARABIDOPSIS ENCODES A RING-H2 UBIQUITIN PROTEIN LIGASE

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The ABI3/VP1 (abscisic acid insensitive 3/viviparous1) family of transcription factors, alternatively activate and repress gene expression during embryogenesis and in vegetative development. In beans and *Arabidopsis*, ABI3 proteins also appear to play a role in chromatin remodeling. A number of protein:protein interactions between ABI3/VP1 family members and other transcription regulators (bZIP, 14-3-3, etc) have been documented1,2. ABI3 Interactive Protein 6 (AIP6) was identified by yeast two-hybrid screening. The deduced AIP6 amino acid sequence revealed the presence of an N-terminal RING-H2 domain characteristic of E3 ubiquitin protein ligases3. While poly-ubiquitylation tags proteins for degradation by the 26S proteasome, mono-ubiquitylation of histones, RNA polymerase II and transcription factors is important for coordinating chromatin structure and transcription during development. A transient increase in AIP6 mRNA levels shortly after germination correlates with the time when ABI3 protein disappears from the seedling. Later in development, AIP6 mRNA is found in most organs and tissues (leaves, root, inflorescence, silique and callus). We are addressing the hypothesis that AIP6-directed ubiquitylation modulates the activity of ABI3, and possibly other factors, towards different promoters, while simultaneously regulating their degradation via the proteasome pathway. Towards that goal, we have constructed epitope-tagged forms of full length AIP6 and ABI3 and shown that they form an *in vivo* complex in yeast (*S. cerevisiae*). This system will be used to map the domains on both proteins that are required for their interaction. We have also demonstrated efficient expression of a cmyc-tagged form of ABI3 in transgenic *Arabidopsis* plants that will be used to study its ubiquitylation and association with specific promoters *in vivo* by chromatin immunoprecipitation (ChIP) analysis.

A LOSS OF FUNCTION ALLELE OF THE *ABI3 INTERACTING PROTEIN 6* GENE (*AIP6*, AT3G05545) CAUSES SEMI DWARFISM AND DELAYED FLOWERING IN *ARABIDOPSIS*

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Plants may respond to metabolic or environmental stress by arresting or delaying development at various times during their life cycle. Genetic studies from various laboratories have implicated the abscisic acid (ABA) transducing factors, ABI3, ABI4 and ABI5, in responses to high sugar-induced stress and ABA. These studies have indicated that ABI3 may function broadly throughout the plant life cycle.[1,2,3] We have identified **ABI3 Interacting Protein 6** (AIP6), encoded by AGI locus At3g05545, as a possible regulator of ABI3 function. The AIP6 protein contains a RING-H2 zinc finger distinctive of a class of eukaryotic ubiquitin protein ligases. We have shown that epitope-tagged, full-length AIP6 and ABI3 forms an *in vivo* complex in yeast (L. Host *et al*, 2005, this meeting). We hypothesize that AIP6 facilitates the A ubiquitylation of ABI3, possibly modifying its biochemical activity and simultaneously triggering its degradation by the 26S proteasome. In order to define the function of AIP6 *in planta*, we have confirmed the presence of a T-DNA insertion within exon 5 of the AIP6 gene in line *aip6-S345*. Plants homozygous for this allele show a complete absence of AIP6 mRNA, indicating a loss-of-function mutation. Homozygous progeny display a semi-dwarf phenotype (~50% reduction in leaf and plant size) and significant delay in flowering, when compared to the wild-type (Col-O) control. Both alterations suggest an involvement of AIP6 in a gibberellic acid (GA) – meditated mechanism, which would be consistent with the antagonistic roles of GA and ABA. Current experiments are aimed at further exploring the role of AIP6 in GA-promotion of flowering.

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EXPRESSION OF TYPE II MADS-BOX GENES DURING POPLAR BUD DORMANCY

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Perennial growth, wood formation and bud dormancy are traits that distinguish trees from annual herbaceous plants. Bud dormancy is an adaptive trait allowing survival during unfavorable environmental conditions. In *Populus,* the vegetative bud is composed of the shoot meristem, embryonic leaves and stipules enclosed by two or more pairs of bud scales. In some ways vegetative bud development and dormancy appear to be similar to flower initiation and development. For example, bud development is a photoperiod response and dormant buds require chilling temperatures to resume growth. Because of these similar features to floral initiation and development, we were interested if analogous regulatory factors govern bud development and dormancy in poplar. Since MADS-box genes regulate many aspect of flowering, we wanted to determine if any poplar MADS-box genes are expressed in developing vegetative buds of poplar during initiation, development and dormancy.

From *Populus trichocarpa* genome sequence (http://genome.jgi-psf.org/Poptr1/Poptr1.home.html), 274 unique gene models were retrieved that represented 99 unique scaffold locations. Alignment of these gene models with *Arabidopsis* and rice type I and II MADS-box genes were performed using ClustalX1.83. From this, 48 gene models representing putative type II MADS-box genes selected and aligned with 37 known *Arabidopsis* and rice type II MADS-box genes to predict their functional classes.

To determine if type II MADS-box genes are expressed in vegetative buds, degenerate primers for eight gene classes (A, B-AP_3, B-PI, E, F, FLC, G, and SVP) were used for semi-quantitative RT-PCR analyses of mRNA from terminal buds from hybrid poplar *(Populus deltoids* X *Populus trichocarpa*) grown over 8 weeks of short day (SD) followed by 16 weeks of SD and cold (4 °C) treatment. Expression of seven out of eight classes and eleven out of seventeen predicted genes from four classes (A, B-PI, FLC, and SVP) was detected in poplar vegetative buds. *FLC*-like and *SVP*-like genes showed similar expression patterns during poplar vegetative bud dormancy suggesting that analogous regulatory factors might be involved in floral vernalization and vegetative bud break.

CDNA SUBTRACTIVE HYBRIDIZATION INDICATES RAPID GENOMIC RESPONSE OF MALUS TO FIRE BLIGHT CHALLENGE

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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 approach to characterize the functional genomic response of apple over time to infection by *E. amylovora*. In order to overcome the historical limitations associated with cDNA library construction and screening, the analysis of EST clones, and even microarray analysis, subtractive hybridization coupled with suppression PCR was used for a global analysis of gene expression subject to change upon infection. By subtracting cDNAs synthesized from mRNAs expressed in one state from cDNAs derived from mRNAs expressed in another state 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. In this study, the subtractive hybridization approach was used to compare the populations of mRNA in mock inoculated (buffer controls) and *E. amylovora* inoculated 'Gale' apple leaves at time intervals after inoculation for differences in gene expression. Tissue samples were collected within 4 to 6 mm of inoculation site from both buffer and pathogen-challenged leaves. Total RNA was isolated from leaf samples, converted into cDNA, and used as tester (transcripts to be cloned after subtraction) and driver (reference transcripts removed) in both forward (pathogen inoculated = tester) and reverse (control = tester) cDNA hybridization subtraction. Gel electrophoresis of PCR-amplified subtracted cDNAs and unsubtracted controls indicated a greater quantity and size diversity in reverse subtracted (down-regulated) samples collected at 1 h and 2 h in comparison to pooled early samples (15 min, 1 h, 2 h, 6 h, 12 h, and 24 h), pooled later samples (48 h and 72 h), 1 h, 2 h, and 12 h forward subtracted (up-regulated), or 24 h and 48 h reverse subtracted samples. PCR amplified subtracted cDNAs were cloned, sequenced, and identified by BLAST analysis. Several photosynthesis related genes, as well as genes related to other cellular processes, were suppressed 2 h after inoculation, including ribose-1,5 bisphosphate carboxylase small subunit (rbcS), chlorophyll a/b binding protein (CAB), a metallothionein-like protein (MT-3), and endomembrane protein 70 (ENDO-70). Expression of CAB and ENDO-70 were equivalent to controls 6 h and 12 h after inoculation.

ISOLATION AND ANALYSIS OF A CYTOPLASMIC DYNEIN FROM THE SOYBEAN PATHOGEN *HETERODERA GLYCINES*

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The soybean cyst nematode (SCN), *Heterodera glycines*, is the major soybean pest in the U.S., causing over $1 billion in losses annually. We have isolated cytoplasmic dynein from SCN, designated *H. glycines* dynein light chain 1 (HgDLC1), with the hope of expressing it in plants as RNAi constructs to control SCN. Our clone was 270 nt in length and highly homologous to cytoplasmic dynein light chain 1 sequences in *Caenorhabditis elegans*, *Drosophila melanogaster*, *Chlamydomonas reinhardtii*, *Homo sapiens*, and *Mus musculus*. In these species, dynein has been shown to have essential functions for survival, being a necessary component of the multimeric dynein complex involved in moving cargo along microtubules within cells. HgDLC1 contains the cargo-binding domain of dynein and a sequence homologous to the N-myristoylation domain, which is a site for acylation. *In situ* hybridizations using digoxigenin-labeled dynein RNA probes derived from the HgDLC1 cDNA we isolated confirm that dynein is expressed throughout the lifecycle of SCN. We are currently running RT-PCR to determine the levels of dynein expression at certain time points throughout development.

PROTEOMIC ANALYSIS OF B-GONGLYCININ AND GLYCININ IN DIFFERENT SOYBEAN GENOTYPES

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Beta-conglycinin and glycinin are two major storage proteins that together comprise 70 to 80 % of the total protein. In this study, we have investigated variation of β-conglycinin and glycinin (acidic and basic) in cultivated and wild soybean seeds using 2-D gel electrophoresis and mass spectrometry based proteomic techniques. We have chosen four cultivated Glycine max accessions (PI 423954, PI 89138, PI 594777 and PI 59845) and four wild Glycine soja accessions (PI 407027, PI 407282, PI 366120, and PI 393551) and compared the protein variations. Our results showed all wild, G. soja genotypes have total of thirteen β-conglycinin subunits (α subunit of β-conglycinin, α´ subunit of β-conglycinin and β subunit of β-conglycinin) when compared to eleven in cultivated G. max genotypes. The cultivated soybeans had 14-20 acidic and basic subunits of glycinin whereas the wild genotype soybeans contained 19-22 acidic and basic subunits. Our data indicated that there were major variations of protein profiles between cultivated and wild soybean seeds rather than among accessions in the same genotypes.

NEGATIVE-ION MODE NANOELECTROSPRAY TANDEM MASS SPECTROMETRY: POTENTIAL TO IMPROVE THE DETECTION OF PATHOGEN PROTEINS

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Our laboratory is interested in elucidating the proteome of *Uromyces appendiculatus*, the causal agent of common bean rust. Knowledge of the proteins that form uredospores, germlings, appressoria, and haustoria may be used to define host-pathogen interactions. We have produced more than 55,000 tandem mass spectra from *U. appendiculatus*. However, many of the collected spectra are of "poor quality" and the resulting interpretation by Mascot can result in a high occurrence of false positive matches. We hypothesize that experimentation in the negative-ion mode may reduce the likelihood of false positive matches that arise "poor quality" spectra. Negative-ion mode nanoelectrospray mass spectrometry is a very under-utilized tool in the structural characterization of peptides. Its under-utilization stems from the fact that traditional gold-coated nanoelectrospray emitters have a tendency to undergo electrical discharge when operated in the negative-ion mode. Nevertheless, this electrical discharge is not observed when the nanoelectrospray experiment is carried out using a polyaniline-coated nanoelectrospray emitter. Due to different charge localization effects that occur in the negative-ion mode, we have found that many peptide ion fragments are found to be generated exclusively in the negative-ion mode experiment. In addition, negative-ion mode tandem mass spectra contain less chemical background and hence are easier to interpret. We have demonstrated that negative-ion mode nanoelectrospray mass spectrometry has remarkable potential to reduce false positive rates and improve our analysis of the proteome of *U. appendiculatus*.

IDENTIFICATION OF COLD-RESPONSIVE GENES IN BLUEBERRY (*VACCINIUM CORYMBOSUM L.*) USING A SUBTRACTIVE HYBRIDIZATION APPROACH

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The United States is the world's leading blueberry producer. The blueberry industry in the U.S., however, suffers from a lack of cold hardy cultivars. Consequently, there is a need to identify genes associated with cold hardiness and use this information to develop cold hardy blueberry cultivars. Toward this direction, our laboratory previously has been using the Expressed Sequence Tag (EST) approach to identify cold-responsive genes from standard cDNA libraries prepared from flower bud RNA from cold acclimated and non-acclimated plants. However, random picking and sequencing of even several thousand clones from standard cDNA libraries will result in selection of clones representing more highly abundant transcripts because these clones will be present in the libraries at a higher frequency than those representing less abundant transcripts. Important regulatory genes, such as transcription factors, are often expressed at rather low levels and over a shorter timeframe. Thus, they can be missed using this approach. Therefore, here we report on the preparation of subtracted and reverse subtracted libraries using procedures that help to increase the chances of finding rarer classes of transcripts by helping to normalize the distribution of clones. The subtracted library was prepared in such a way to enrich for transcripts that are expressed at higher levels in blueberry flower buds at 400 hours of cold exposure than at 0 hours of cold exposure and vice versa for the reverse subtracted library. Clones from each library have been picked and sequenced and confirm that the libraries are of good quality and enriched for cold-responsive genes. Contig analyses and BLAST searches have been performed to categorize the genes. Quantification of expression of specific cold up-regulated genes using qRT-PCR is in progress.

RUB1 CONJUGASE AND VEGETATIVE BUD DEVELOPMENT

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Vegetative growth of trees is characterized by an active period during spring and summer followed by a quiescent or dormant phase. This dormant phase is an adaptive mechanism ensuring survival during unfavorable environmental conditions encountered in winter. Because of the adaptive significance of bud dormancy and developmental arrest to tree growth and survival, understanding the genetic aspects of bud dormancy is an essential part of understanding tree biology. Using *Populus* (poplar) as a model system, functional genomics and reverse genetic approaches are identifying regulatory genes important to bud development and dormancy. In this study, an EST identified using cDNA-AFLP analysis of gene expression changes during poplar apical bud development were chosen for further study.

Sequence analysis of the EST suggests that it likely encode an RUB1 conjugase-like enzyme. RUB1 conjugase is an important component of Ubiquitin/26S proteasome pathway. Components of this pathway are involved in almost all aspects of plant development including cell cycle, hormone signaling and disease resistance. As a first step in understanding the potential involvement of RUB1 conjugase and Cullin1 proteins in bud development and dormancy, spatial and temporal analysis of gene expression was performed. In addition to determine if bud development and dormancy induction is associated with changes in protein ubiquination, changes in RUB1 and Ubiquitin protein conjugates were analyzed. The results of expression and protein analysis suggest that components of Ub/26S proteasome pathway may play an important role in bud development.

MORPHOLOGICAL AND PHYSIOLOGICAL ALTERATIONS IN TRANSGENIC TOBACCO (*NICOTIANA TABACUM* L) DUE TO THE ECTOPIC EXPRESSION OF *ARABIDOPSIS THALIANA* AND *BRASSICA NAPUS BABY BOOM* GENES

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Gain-of-function studies in Arabidopsis (*Arabidopsis thaliana*) and oilseed rape (*Brassica napus*) showed that the AP2/ERF domain transcription factor gene BABY BOOM (BBM) activates cell proliferation and morphogenesis pathways leading to spontaneous organogenesis and somatic embryogenesis. Here we examined the effect of ectopically expressing the *Arabidopsis* and oilseed rape *BABY BOOM (BBM)* genes on tobacco (*Nicotiana tabacum* L.) development. Transgenic tobacco lines expressing the *BBM* genes under the control of Cauliflower Mosaic Virus (CaMV) 35S promoter showed a similar range of vegetative and reproductive defects as previously observed in 35S::BBM *Arabidopsis* and oilseed rape transgenics.

Two phenotypic classes were observed among the transgenic lines. The moderate phenotypic class were plants of normal stature, which showed mild leaf rumpling and floral organ defects. Plants in this group were fully fertile or showed reduced fertility. The severe class of 35S::BBM transgenics were extremely slow growing, exhibited extreme leaf rumpling and floral organ defects, and were fully male and female sterile. Morphological and ultrastructural observations showed that compared to wild-type plants, transgenic tobacco show a reduced number of leaf trichomes, a reduction in the number of leaf cell layers and chloroplasts per cell, and an increase in starch accumulation in leaf mesophyll cells. Leaves of 35S::*BBM* tobacco had a lower photosynthetic activity than wild-type plants. Floral organ abnormalities included differential organ growth and male and female gametophyte abortion. The regeneration capacity of the 35S::BBM transgenics was also examined. Neither spontaneous somatic embryogenesis nor enhanced regeneration capacity were observed in the moderate lines, however the severe 35S::BBM lines showed spontaneous shoot formation in seedlings after propagation through haploid embryo culture. Inducible post-translational activation of a 35S::BBM:GR (rat glucorticoid receptor) fusion protein was used to obtain fertile tobacco transgenics with severe over-expression phenotypes. Spontaneous root and shoot formation was observed in these lines after treatment with dexamethasone inducer.

ISOLATION AND CHARACTERIZATION OF THE SOYBEAN CYST NEMATODE (*HETERODERA GLYCINES*) HOMOLOG OF *CAENORHABDITIS ELEGANS UNC-97* GENE

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The soybean cyst nematode (SCN) is the major pest of soybean in the United States and no agronomically important soybean cultivars are resistant to all races of SCN. Our major goal is to develop new modes of soybean resistance to SCN using biotechnology. Therefore, we identified and characterized the SCN (*Heterodera glycines*) homolog of the *Caenorhabditis elegans unc-97* gene (*Hg-unc97*) a gene that may be essential for SCN survival. In *C. elegans* the *unc-97* gene encodes a protein involved in movement that constitutes a component of the muscular adherens junction by interacting with the β-integrin PAT-3 at the focal adhesion-like attachment sites of muscles. In *C. elegans* mutants of *unc-97* are limp, egg laying-defective and paralyzed. We identified a SCN partial cDNA clone, *Hg-unc97*, with high amino acid identity to *C. elegans unc-97* and used PCR to obtain the full-length *Hg-unc97* gene. The *Hg-unc-97* cDNA is 1574 nt, encoding a 522 aa protein. Phylogenetic analysis of the full-length sequence revealed 63% amino acid identity with *C. elegans unc-97*. Real-time reverse transcription polymerase chain reaction (RT-PCR) indicated that *Hg-unc-97* is expressed in all stages of the SCN life cycle. The relative ratios of *Hg-unc97* between the different time points in the life cycle obtained by RT-PCR indicated a 6-fold increase in *Hg-unc-97* RNA in J2s, respective to eggs, and nematodes 15 and 30-day post infection. The use of the *Hg-unc-97* to broaden resistance of soybean to SCN will be tested in future experiments.

PLANT PROJECTS IN THE ENTREZ GENOME PROJECT DATABASE

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The Entrez **Genome Project** database is a new database that has been added to the Entrez system at NCBI. It is a collection of large-scale sequencing, assembly, annotation, and mapping projects. It is organized into organism specific overview pages from which all projects related to the organism can be retrieved. Each overview page provides status of each project, links to project data in other Entrez databases, provides relevant publications, and links to internal and external resources associated with the project. Currently there are 56 plants in the database that have 21 genome sequencing projects, 14 BAC end sequencing projects, 36 large scale EST projects, and 12 mapping projects associated with them. A detailed description of the plant projects in the Genome Project database will be provided.

Visit: Entrez Genome Project database at
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj

FUNGAL RESPONSE TO PLANT DEFENSE COMPOUNDS: A TWO-HYBRID APPROACH TO DISSECT THE SIGNALTRANSDUCTION CASCADE

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One of the ways by which plants defend themselves against pathogens is by producing a variety of fungistatic compounds like phytoalexins and/or phytoanticipins. For example, pisatin is an anti-fungal defense compound uniquely produced by the garden pea, *Pisum sativum*. However, pisatin can also trigger a counter defense pathway in the fungus *Fusarium solani*, a virulent pea pathogen. Pisatin is detoxified in *F. solani* by a cytochrome P450 monooxygenase, pisatin demethylase. The gene encoding the monooxygenase, PDA1, has been found to be strongly induced by pisatin. A 40 bp pisatin-reponsive element has been identified within the promoter sequence of PDA1. This element serves as a binding site for the binuclear zinc transcription factor PRF (**P**isatin **R**esponse **F**actor) also identified in the lab. PRF appears to mediate a signal transduction pathway in the fungus that responds to the stress induced by pisatin. What sort of signal transduction pathway does pisatin initiate in the fungus? Where and how does PRF act in this pathway? To answer these questions, we are currently identifying proteins that interact with PRF. Yeast two hybrid analysis is being performed with PRF as the prey and the cDNA library of *F. solani* expressed as bait. Interacting proteins could include: a) coactivators and proteins which facilitate DNA binding or transcriptional regulation, b) proteins that function upstream of PRF in well-known or unknown stress pathways. The two-hybrid analysis has revealed two promising proteins showing high levels of interaction with PRF – V25 and V27. V25 is a novel protein and similar to one of the two domains of Mn/Fe superoxide dismutase. The protein is predicted to possess a mitochondrial targeting sequence and a nuclear localization signal. Another positive clone, V27, was found to have a nuclear localization signal. Currently, binding studies are underway to establish an *in vitro* interaction between V25 and PRF. In addition, we are also trying to understand how PRF responds to pisatin in *Fusarium*. We are using Western Blots to detect changes in post-translational modification and cellular localization of PRF in *Fusarium*. Preliminary evidence, point to the occurrence post-translational modifications in vivo. At present, we are trying to define the nature of these modifications. Characterization of this pathway will thus enable a better understanding of the fungal infection process and the consequent root rot caused in pea plant cultivations.

EFFECT OF ETHYLENE AND ITS INHIBITORS ON THE NUMBER OF SOYBEAN CYST NEMATODE IN SOYBEAN CYST NEMATODE–INFECTED SOYBEAN ROOTS

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Excised soybean roots infected with soybean cyst nematode *Heterodera glycines* produced ethylene at 2-4 times the rate of non-infected roots when ethylene was measured 7 days post inoculation. The number of nematodes that matured on the infected roots was increased by approximately 60% when the roots were exposed to 1 ul/L ethylene. The number of mature female nematodes was 90% inhibited when the ethylene action inhibitors 1-methylcyclopropene (MCP) or 2,5-norbornadiene (NBD) was applied to the infected roots. Moreover, the number of nematodes that matured on the roots of the ethylene insensitive soybean mutant ETR1-1 was 40% less than that of the wild type. Our results indicate that ethylene positively affects the parasitism of soybean cyst nematode *H. glycines* in soybean roots. This suggests that this plant hormone might play an important role in the development of the feeding structure (syncytium) of *H. glycines* in infected soybean roots.

SEPARATION AND IDENTIFICATION OF SOYBEAN LEAF PROTEINS BY 2D-PAGE AND MASS SPECTROMETRY

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To establish a proteomic reference map for soybean leaves, we separated and identified leaf proteins using two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) and mass spectrometry (MS). We detected approximately 700 protein spots by 2D-PAGE using a pH 3-10 immobilized pH gradient (IPG) strip and visualization with colloidal Coomassie brilliant blue (CBB) G-250. Tryptic digests of approximately 256 protein spots were subjected to peptide mass fingerprinting by matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry. More than 30 protein spots were identified searching against NCBInr and SwissProt databases using the Mascot search engine. Twenty spots that were not identified by MALDI-TOF analysis were analyzed by liquid chromatography mass spectrometry (LC- MS) and were identified by searching against the NCBInr and expressed sequence tags (EST) databases. Many abundant leaf proteins are present in multiple spots. These results indicate that 2D-PAGE, combined with MALDI-TOF and LC- MS, is a sensitive and powerful technique for protein separation and identification of soybean leaf protein.

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