Mid Atlantic Plant Molecular Biology Society

21st Annual Meeting

National Wildlife Visitors Center
Pautuxent Research Refuge

August 19th & 20th
2004

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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 2004. 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, postdocs, 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
Chair

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2004 MAPMBS MEETING SCHEDULE

Thursday, August 19

9:30 am Registration and Poster set-up

9:40 am Introductions

Session I Human Health: Biosecurity and Nutritional Quality
Moderator: Bret Cooper, Soybean Genomics and Improvement Lab, USDA-ARS, Beltsville

9:45 am **Philip Berger**, USDA-APHIS-PPQ
APHIS-PPQ: New detection technologies and biosecurity

10:15 am **Joseph M. Chiera**, Department of Plant Pathology, Physiology, and Weed Science Virginia Polytechnic Institute and State University, Blacksburg, VA
Engineering soybean to reduce seed phytate content

10:35 am Break – Coffee, Exhibitors, Poster Set-up and Viewing

11:00 am **Howard Damude**, Pioneer Crop Genetics Research, DuPont Experimental Station, Wilmington DE
Improving the health properties of soy foods by the genetic modification of soybean plants

Session II Global Responses to Plant Biotic and Abiotic Stress
Moderator: Jeannie Rowland, Fruit Lab, USDA-ARS, Beltsville

11:30 am **Tara VanToai,** USDA-ARS Soil Drainage Research Unit, Columbus, OH
Gene expression phenotypes associated with flooding tolerance of SAG12:IPT Arabidopsis

11:50 am Business Meeting

12:00 pm Lunch – Exhibitors and Poster Viewing

1:00 pm **Jane Glazebrook**, Department of Plant Biology, University of Minnesota
Comparison of Arabidopsis responses to Alternaria brassicicola and Pseudomonas syringae by expression profiling

1:30 pm **Fenglong Liu**, The Institute for Genomic Research, Rockville, MD
Transcriptional profiling of hypoxic stress response in Arabidopsis

1:50 pm **Fumiaki Katagiri**, Dept. of Plant Biology, Univ. of Minnesota Center for Microbial and Plant Genomics
Plant disease resistance: insights from global expression profiling

2:20 pm **Robert E. Farrell Jr.**, Department of Biology, Pennsylvania State University, York, PA
Analysis of genes expressed in response to cold temperatures under different photoperiods in peach bark

2:40 pm **Anastasia Nikolskaya**, Protein Information Resource and Georgetown University Medical Center
PIRSF protein family classification system and protein sequence annotation

3:00 pm Break – Exhibitors, Poster Viewing

KEYNOTE ADDRESS

Introduction: Leslie Wanner, USDA-ARS, Vegetable Laboratory, Beltsville, MD

3:30 pm **Michael Thomashow**, MSU-DOE Plant Research Laboratory
Gene Regulons and Regulatory Circuits Involved in Plant Cold Acclimation

4:30 pm Close of day

Friday, August 20

Session III Comparative Genomics and Functional Annotation
Moderator: June Kwak, Dept. Cell Biology and Molecular Genetics, University of Maryland

9:30 am **Blake Meyers**, University of Delaware, Delaware Biotechnology Institute
Whole-genome transcriptional analysis of Arabidopsis using massively-parallel signature sequencing (MPSS)

10:00 am **Nadim W. Alkharouf**, USDA-ARS, Soybean Genomics and Improvement Laboratory, Beltsville, MD
The use of data warehousing and online analytical processing (OLAP) to identify resistance genes and pathways in a resistant soybean cultivar (Peking) against the cyst nematode

10:20 am Break – Exhibitors and Poster Viewing, Coffee

10:50 am **Antoni Rafalski**, DuPont Crop Genetics, Wilmington, DE
Maize Genome Structure, Linkage Disequilibrium and Genetic Association Mapping.

11:20 am **Yong-Li Xiao**, The Institute for Genomic Research, Rockville, MD
High Throughput sequencing and analyzing of the cDNAs of hypothetical genes of Arabidopsis on chromosome 2

11:40 am **Chris Town**, The Institute for Genomic Research, Rockville, MD
The Medicago truncatula genome sequencing project: Strategy, status and results

12:10 pm LUNCH: Exhibitors and Poster Viewing

SESSION IV MOLECULAR STUDIES OF GENE EXPRESSION
Moderator: Nadim Alkharouf, USDA-ARS, Soybean Genomics and Improvement Laboratory, Beltsville, MD

1:10 pm **Rick Amasino**, Dept. Biochemistry, University of Wisconsin, Madison, WI
Vernalization and the epigenetic memory of winter

1:40 pm **June M. Kwak**, Dept. Cell Biology and Molecular Genetics, University of Maryland, College Park, MD

Dissection of guard cell ABA signal transduction mechanisms using combined single cell-type functional genomics and cell biological approaches

2:00 pm **Heven Sze**, University of Maryland, College Park, MD
Expression patterns of a novel AtCHX gene family highlight potential roles in osmotic adjustment and K+ homeostasis in pollen development

2:20 pm **Vincent Klink**, USDA-ARS, Soybean Genomics and Improvement Laboratory, Beltsville, MD
Laser capture microdissection (LCM) and expressed sequence tag analysis of the syncytium formed by the soybean cyst nematode Heterodera glycines in Glycine max (soybean)

2:40 pm **Mallikarjun Lalgondar**, Hyun-Young You, David R. Bevan, and A. Esen, Virginia Polytechnic Institute & State University, Blacksburg, VA 24061
The specific intraction between maize β-glucosidases and a chimeric protein containing dirigent and lectin domains

3:00 pm **Closing Remarks - Adjourn - Take Down Posters**

2004 MAPMBS Poster Session

8 40 **Faten Deeb and Stephen M. Wolniak**, Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD
Kinesin and spermatid differentiation in Marsilia vestita

9 41 **Ping Xue, Mark L. Tucker, Anjana Raina, Mindy L. Ehrenfried, Vanessa K. Thai,** Soybean Genomics and Improvement Laboratory, Plant Sciences Institute, Beltsville Agricultural Research Center, U.S. Department of Agriculture, Beltsville, MD 20705-2350, USA
Sequence and Membrane Array Analysis of Two Subtraction cDNA Libraries for Nematode Infected Soybean Roots

10 42 **Raymond W.M. Fung[a], Chien Y. Wang[a,*], David L. Smith[a], Kenneth C. Gross[a], Yang Tao[b], Meisheng Tian[c],** [a]Produce Quality and Safety Laboratory, Plant Sciences Institute, Beltsville Agricultural Research Center, U.S. Department of Agriculture, Beltsville, MD 20705-2350, USA; [b]Biological Resources Engineering Department, Animal Sciences and Agriculture Engineering, Bldg 142, College Park, MD 20742-2315 USA; [c]HortResearch, Mt Albert Research Centre, Private Bag 92169, Auckland, New Zealand,
Characterization of AOX gene expression in response to MESA and MEJA pretreatment and low temperature in pink tomatoes (Lycopersicon esculentum)

11 43 **Marcelo P. Giovanini[1]; David Puthoff[2, 3]; Herbert W. Ohm[1] and Christie E. Williams[2, 3,4],** [1]Department of Agronomy, Purdue University, 915 W. State St., West Lafayette, IN 47907; [2]USDA-ARS Crop Production and Pest Control Research Unit, West Lafayette, IN 47907; [3]Department of Entomology Purdue University 901 W. State St. West Lafayette, IN 47907
Identification and characterization of a battery of wheat genes regulated BY Hessian fly larval feeding

12 44 **Benjamin Matthews[1,2], Vincent Klink[1], Margaret MacDonald[1], and Nadim Alkharouf [1,2],** [1]USDA, ARS, Plant Science Institute, Soybean Genomics & Improvement Laboratory, BARC-West, Beltsville, MD 20705, USA; [2]School of Computational Sciences, George Mason University, Manassas, VA
Laser capture microdissection and isolation of soybean syncytial cells formed by the soybean cyst nematode

13 45 **Dapeng Zhang, Emily Leamy, Sue Mischke, Michel Boccara* and David.R. Butler***, Alternate Crops and Systems Lab, Plant Sciences Institute, Beltsville Agricultural Research Center, USDA/ARS, Beltsville, MD 20705. USA; *International Cocoa

Genebank, Trinidad, Cocoa Research Unit, University of the West Indies, St. Augustine, Trinidad & Tobago.
Verification of genetic identity in Theobroma cacao germplasm using microsatellite markers

14 46 **Xianying Wei, Anastasia N. Nikolskaya, Cathy H. Wu**, Protein Information Resource, Georgetown University Medical Center
Family classification and integrative analysis of molybdenum cofactor biosynthesis proteins and related proteins

15 47 **Vijayakala Vydeeswaran**, Dept of Cell Biology and Molecular Genetics, 2221 H J Patterson Bldg, Univ. of Maryland, College Park, MD
Fungal Response to Plant Defense Compounds: A Two-hybrid Approach to Dissect the Signal Transduction Cascade

16 48 **James J. Polashock[1] and Peter V. Oudemans[2]** USDA-ARS Fruit Lab[1] and Rutgers University[2]; 125A Lake Oswego Rd, Chatsworth, NJ 08019
Molecular identification of the 'BLACK SHADOW' pathogen of blueberry

17 49 **Xiyan Li[a], Jeffrey F. Harper[b], Shawn Romanowsky[b], Heven Sze[a],** [a]Dept. of Cell Biology & Molecular Genetics, University of Maryland, College Park, MD 20742; [b]Dept. of Biochemistry, University of Nevada, Reno, NV 89557
Calcium ATPase AtECA3 shows tissue-specific expression and supports root and pollen tube growth

18 50 **Nicole G. Novak and Susan D. Lawrence**. Insect Biocontrol Laboratory, USDA-ARS, BARC-West, Building 010A, Room 214, Beltsville, MD 20705
Insect herbivory and volicitin induce genes involved in volatile production

19 51 **José R. Vidal, Julie R. Kikkert and Bruce I. Reisch.** New York State Agricultural Experiment Station, Cornell University, Geneva, NY
Comparative transgene expression in 'Chardonnay' between minimal cassette and circular plasmid transformation

20 52 **Yong-Li Xiao, Shannon R Smith, Nadeeza Ishmael, Julia Redman, Nihkil Kumar, Erin Monaghan, Mulu Ayele, Brian Haas, Christopher D Town,** The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850

High throughput sequencing and analyzing of the cDNAs of hypothetical genes of Arabidopsis on chromosome 2

21 53 **Young Ock Ahn[1,2], Masaharu Mizutani[1], Asim Esen[2], and Kanzo Sakata**[1], [1]Institute for Chemical Research, Kyoto University, Japan[2;] Department of Biology Virginia Polytechnic Institute & State University, Blacksburg, VA 24061
Cloning and functional expression of vicianin hydrolase, a cyanogenic glycosidase, from Vicia angustifolia

22 54 **Heven Sze, Senthilkumar Padmanaban, Kevin W. Bock & Xiyan Li**, University of Maryland, College Park, MD 20742-5815, USA **Françoise Cellier & Genevieve Conéjéro**, INRA/CNRS/AgroM/ UMII, Montpellier, France, **David Honys**, Institute of Experimental Botany AS CR, Praha, Czech Republic; **Ning-Hui Cheng & Kendal D. Hirschi**, Baylor College of Medicine, Houston, TX, USA **David Twell**, University of Leicester, Leicester LE1 7RH, England **John M. Ward**, University of Minnesota, St. Paul, MN, USA
Expression patterns of a novel AtCHX gene family highlight potential roles in osmotic adjustment and K^+ homeostasis in pollen development

Speaker Abstracts

APHIS-PPQ: NEW DETECTION TECHNOLOGIES AND BIOSECURITY

Philip Berger

USDA-APHIS-PPQ Center for Plant Health Science and Technology, Raleigh, NC 27606. Philip.h.berger@aphis.usda.gov

Our agricultural and plant resources are vulnerable to intentional or unintentional introduction of pest and pathogens. The responsibility for protecting US plant resources lies with APHIS as well as DHS, but also involves many universities, state agencies, and private organizations. Effective detection and identification requires both field-deployable and laboratory-based diagnostics that are rapid, sensitive, and inexpensive. We face significant challenges, however, in meeting our safeguarding mission. Unlike the analogous situation for medical or veterinary diagnostics, we require diagnostics for thousands of pathogens and pests, affecting hundreds of hosts. There are nearly 400 species of pest or pathogen on the USDA-APHIS regulated pest list, and hundreds more that are reportable or actionable. In any given year, there can be 10-15 serious plant disease outbreaks affecting major crops in different agricultural regions. At times identification to species is adequate, but frequently we require identification to race, biovar or strain. At times determining geographic origin of an agent is important. We currently use methods based on detection of nucleic acids, immunological methods, and 'classical' methods. Case studies will be presented to illustrate the successes and challenges of integrating sound science, technology, and regulatory policy or actions.

ENGINEERING SOYBEAN TO REDUCE SEED PHYTATE CONTENT

Joseph M. Chiera[1], John Finer[2] and Elizabeth A. Grabau[1]

[1]Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061; [2]Department of Horticulture and Crop Science, OARDC/The Ohio State University, Wooster, OH 44691.
jmchiera@vt.edu

Phytate, the storage form of seed phosphorus, comprises more than 1% of seed weight and more than 60% of total seed phosphorus. Phytate affects the nutritional quality of agriculturally important crops such as soybean (*Glycine max*). Soybean meal, which is used for its protein content, contributes phytate to animal feed. From a nutritional aspect, phytate is poorly digested by non-ruminants, which results in the excretion of phosphorus and the associated environmental phosphorus pollution. In addition, phytate is considered to be an anti-nutrient due to its ability to bind and reduce the bioavailability of mineral cations such as iron and zinc. In seedlings, phosphorus required during germination is released from phytate by the activity of the enzyme phytase.

A transgenic approach was taken to reduce phytate in mature soybean seed. The soybean phytase gene (*GmPhy*) was expressed during seed development to degrade accumulating phytate in the seed. Embryogenic soybean cultures were transformed with an expression vector containing the soybean phytase cDNA controlled by the seed-specific β-conglycinin promoter (α'-subunit). A low copy transformant was recovered and progeny were grown for three additional generations. Developing T_2 and T_3 seeds from transgenic plants were analyzed for phytase activity and mRNA, respectively. Mature T_4 seed was tested for available P and phytate content. Phytase expression during seed development led to an average a 3-fold increase in available P and an 8% reduction in phytate content. Expression of phytase during seed development offers a promising strategy for improving phosphorus availability while reducing phytate content in soybean seed.

Improving the health properties of soy foods by the genetic modification of soybean plants

Howard Damude and Anthony J. Kinney

Pioneer Crop Genetics Research, DuPont Experimental Station, Wilmington DE 19880-0402 USA

Many people have increased their consumption of soy-based foods in recent years in response to the perceived health benefits from consuming soybean protein. Research attention has been focused on the oil component of the soybean to provide health benefits that might complement the positive effect of soy protein in the diet. Initial approaches were based on the concept of changing the existing ratios of the fatty acids in the plant triacylglycerol to make healthier cooking oils. The result has been the production of soybean oils with reduced saturated fatty acid content and with a greatly reduced acid ratio of omega-6 to omega-3 fatty acids. In addition to improving the balance of polyunsaturated acids in the diet, this latter oil is oxidatively stable and can be used as a substitute for partially hydrogenated oils in many foods thus helping eliminate *trans* fatty acids from the diet. More recent efforts to mine the biodiversity of the plant and microbe kingdoms have uncovered new genes that can be used to produce novel fatty acids in soybean oil. These new fatty acids may provide a source of health-promoting ingredients for soy protein-based foods. Examples of these new fatty acids include conjugated fatty acids and long-chain omega-3 polyunsaturated fatty acids.

GENE EXPRESSION PHENOTYPES ASSOCIATED WITH FLOODING TOLERANCE OF *SAG12:IPT* ARABIDOPSIS

Tara VanToai[1], Fenglong Liu[2], Linda Moy[2], Le Nguyen Huynh[3], Geoffrey Bock[2], Gary Banowetz[4], and John Quackenbush[2]

[1]USDA-ARS-Soil Drainage Research Unit, Columbus, OH. [2]The Institute for Genomic Research, Rockville, MD; [3]Department of Horticulture and Crop Science, The Ohio State University, Columbus, OH. [4]USDA-ARS National Forage Seed Production Research Center, Corvallis, OR

Transgenic *Arabidopsis* plants (IPT) containing the *SAG12:ipt* auto-regulated cytokinin production capability accumulate more cytokinin and remain greener and produce more biomass and seeds than wild-type (WT) plants under flooding stress. To gain insight into changes in gene expression that contribute to flooding tolerance, whole genome microarray analysis of the 27,000 *Arabidopsis* genes was conducted at specific times during a 24-hour period of non-flooded control, water-logging and complete submergence. Hierarchical clustering using Euclidean distance metric revealed distinct patterns of gene expression in these treatments. Under stresses, genes of photosynthesis and energy utilization pathways were expressed 2- to 4-fold more in the tolerant IPT plants than in WT plants suggesting that flooding tolerance mechanisms involve both energy production and utilization. Transcript levels of genes involved in transport (oxygen, carbohydrates, lipids, and hormones), disease resistance, stress protection and AP2/EREBP transcription factor increased significantly in IPT plants under submergence stress, but not in WT plants. These cytokinin responsive genes probably play with a critical role in delaying plant senescence and improving flooding tolerance.

COMPARISON OF ARABIDOPSIS RESPONSES TO *ALTERNARIA BRASSICICOLA* AND *PSEUDOMONAS SYRINGAE* BY EXPRESSION PROFILING

S. van Wees[1], S. Goregaoker[1], and J. Glazebrook[1,2]

[1]Torrey Mesa Research Institute, 3115 Merryfield Row, San Diego, CA 92121 (closed); [2]Department of Plant Biology, University of Minnesota, 1445 Gortner Avenue, St. Paul, MN 55108

Biotrophic plant pathogens grow in living tissue, while necrotrophic pathogens first kill host tissue, and subsist on the remains. Plant resistance to biotrophic pathogens often involves gene-for-gene resistance and salicylic acid (SA)-mediated signaling. In contrast, gene-for-gene resistance relationships have not been identified for necrotrophic pathogens, and SA-dependent defense responses are generally ineffective. Rather, defense responses controlled by jasmonic acid (JA) and ethylene (ET) are effective in some cases. We have studied resistance to necrotrophs using the fungal pathogen *Alternaria brassicicola* strain MUCL20297, which is a poor pathogen of Arabidopsis, failing to cause disease symptoms on any accession tested. Jasmonic acid signaling and the Arabidopsis phytoalexin, camalexin, are required for resistance, but salicylic acid signaling is not required. In contrast, resistance to the bacterial pathogen *Pseudomonas syringae* requires salicylate signaling but not jasmonic acid signaling or camalexin. Plant responses to these two pathogens were compared by expression profiling using an Affymetrix array representing one-third of the Arabidopsis genome. Gene expression changes occurred within 12 hours after *Alternaria* treatment. Approximately 50% of *Alternaria*-induced genes were also induced by *P. syringae*. Reverse genetics analysis of *Alternaria*-induced genes led to discovery of a cytochrome P450 monooxygenase required for camalexin synthesis and resistance to *Alternaria*.

TRANSCRIPTIONAL PROFILING OF HYPOXIC STRESS RESPONSE IN ARABIDOPSIS

Fenglong Liu[1], Tara VanToai[2], Linda Moy[1], Geoffrey Bock[1], Lara Linford[1], John Quackenbush[1, 3, 4]

[1]The Institute for Genomic Research, Rockville, MD; [2]USDA-ARS-Soil Drainage Research Unit, Columbus, OH; [3]Department of Biochemistry, George Washington University, Washington, DC; [4]Department of Chemical Engineering, University of Maryland, College Park, MD; fliu@tigr.org

Plants have evolved adaptation mechanisms to sense oxygen deficiency in their environment and make coordinated physiological and structural adjustments to enhance their hypoxic tolerance. To gain insight into how plants respond to low oxygen stress, gene expression profiling was carried out at 9 time points over 24 hours, in wild-type and flooding-tolerant P_{SAG12}*:ipt Arabidopsis* plants under normoxic and hypoxic conditions, using whole-genomic DNA amplicon microarrays. Transcript level of genes involved in glycolysis and fermentation pathways, ethylene synthesis and perception, calcium signaling, nitrogen utilization, trehalose metabolism, and alkaloid synthesis was significantly altered in response to oxygen limitation. Analysis based on gene ontology (GO) terms suggested a significant down-regulation of genes whose functions are associated with cell walls, nucleotide structures, water channels and ion transporters, and a significant up-regulation of genes involved in transcriptional regulation and auxin responses under conditions of oxygen shortage. Promoter analysis on a cluster of up-regulated genes revealed a significant over-representation of the AtMYB2 binding motif (GT-motif), a sugar response element (SURE)-like motif and a G-box-related sequence, and also identified several putative anaerobic response elements. Finally, the real time PCR using 29 selected genes independently verified the microarray results. The study represents one of the most extensive studies conducted to date investigating the hypoxia-perturbed transcriptional networks in plants.

PLANT DISEASE RESISTANCE: INSIGHTS FROM GLOBAL EXPRESSION PROFILING

Fumiaki Katagiri

Dept. of Plant Biology, Univ. of Minnesota, Center for Microbial and Plant Genomics

As a result of the widespread application of global mRNA expression profiling technology in the field of plant-pathogen interactions, we can now easily appreciate the magnitude and complexity of the plant response to pathogen infection. During resistant responses after infection of *Pseudomonas syringae* strains, more than 2000 genes among 8000 genes monitored substantially changed their mRNA levels within 9 hours in Arabidopsis leaves. Differences in gene expression patterns during resistance and susceptible responses could be mainly explained by quantitative and/or kinetic differences in expression changes. These observations suggest the following notions. (1) Plant defense responses are not highly specialized to a particular pathogen. Rather many defense mechanisms are turned on, and some of the mechanisms could be effective against a particular pathogen. This notion is supported by other expression profiling works that demonstrated substantial overlaps in expression changes during interactions with very different pathogens. (2) Regulatory mechanisms for gene expression changes are largely shared in resistant and susceptible responses. This notion is supported by the fact that mutations in many regulatory genes affect both resistant and susceptible responses. (3) Stronger and/or faster induction of a similar set of defense mechanisms is crucial in expressing resistance to certain pathogens. These notions further suggest that the regulatory mechanism for the response to pathogens may not be well understood if we employ a model with relatively independent signaling pathways specific to particular phenotype.

ANALYSIS OF GENES EXPRESSED IN RESPONSE TO COLD TEMPERATURES UNDER DIFFERENT PHOTOPERIODS IN PEACH BARK

Robert E. Farrell, Jr.[1], Carole L. Bassett[2], Timothy S. Artlip[2], and Michael E. Wisniewski[2]

[1]Department of Biology, Pennsylvania State University, York, PA 17403
[2]USDA-ARS, Appalachian Fruit Research Station, Kearneysville, WV 25430

The behavior of all cells and their response to environmental change is governed by the induction and repression of various groups of genes. Global approaches to identifying genes that are modulated in response to stress have been successfully applied to several plant and animal systems. However, certain limitations restrict the degree to which each approach is successful in documenting true differences in expression. For example, microarray analysis is restricted to previously isolated genes and does not allow identification of unique, undiscovered genes that might be crucial to the response being studied. EST library approaches overcome this problem in part, but are labor-intensive and tend to be somewhat biased for genes that are moderately-to-highly abundant. One approach that overcomes these limitations is the synthesis of gene libraries by subtractive hybridization and cloning. 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. By varying which cDNA serves as the driver of the hybridization reaction and which cDNA serves as the tester, one can obtain both up-regulated (forward subtraction) and down-regulated (reverse subtraction) sequences in response to a defined set of experimental conditions. In an effort to profile gene expression at different temperatures under different photoperiods, we have created subtracted libraries from peach (*Prunus persica*) bark tissues sampled from trees maintained at 5° and 25° under a short day (SD) photoperiod or exposed to a night break (NB) interruption during the dark period of the SD cycle. Differentially expressed sequences enriched by performing forward and reverse subtractions using various combinations of temperature and photoperiod treatments were cloned, sequenced, and identified by BLAST analysis. The results have been analyzed relative to known or predicted functions of the gene products identified and their association with the various experimental manipulations.

PIRSF PROTEIN FAMILY CLASSIFICATION SYSTEM AND PROTEIN SEQUENCE ANNOTATION

Anastasia N. Nikolskaya
Protein Information Resource, Georgetown University Medical Center, Washington, DC ann2@georgetown.edu

High-throughput genome projects have resulted in a rapid accumulation of predicted protein sequences; however, experimentally verified information on protein function lags far behind. The common approach to inferring function of uncharacterized proteins based on sequence similarity to annotated proteins often results in over-identification, under-identification, or even mis-annotation. To facilitate accurate, consistent and rich functional annotation of proteins, Protein Information Resource (PIR, http://pir.georgetown.edu/) employs a classification-driven rule-based automated annotation method supported by a bioinformatics framework that provides data integration and associative analysis.

Towards this goal, PIR has developed the SuperFamily (PIRSF, http://pir.georgetown.edu/pirsf/) classification system. This classification, based on the evolutionary relationships of whole proteins, allows annotation of both specific biological and generic biochemical functions. The system adopts a network structure for protein classification from superfamily to subfamily levels. The primary PIRSF classification unit is the *homeomorphic family* whose members are *homologous* (sharing common ancestry) and *homeomorphic* (sharing full-length sequence similarity with common domain architecture). The PIRSF database consists of two data sets: preliminary computer-generated clusters and curated families. Families are curated for name, membership, parent-child relationships, domain architecture, and optional description and bibliography.

When an experiment yields a sequence (or a set of sequences), it is often necessary to assess the function of this protein based on sequence alone. The quality of this assessment is often critical for interpreting experimental results and making hypothesis for future experiments. Searching a protein sequence against curated PIRSFs provides faster and more accurate results than a BLAST search against an uncurated protein database because it avoids the pitfalls such as numerous erroneous annotations in the databases, best hits based on a domain secondary to the protein function, spurious hits etc. PIRSF allows *going from sequence to function* and getting curated, reliable and enriched information. The integrative approach leads to novel prediction and functional inference for uncharacterized proteins, allows systematic detection of genome annotation errors, and provides sensible propagation and standardization of protein annotation.

PIR recently joined the European Bioinformatics Institute and Swiss Institute of Bioinformatics to establish UniProt (http://www.pir.uniprot.org/), an international resource of centralized, value-added protein knowledge that unifies PIR, Swiss-Prot, and TrEMBL databases. PIRSF is an integral part of the UniProt annotation pipeline.

GENE REGULONS AND REGULATORY CIRCUITS INVOLVED IN PLANT COLD ACCLIMATION

Michael F. Thomashow

MSU-DOE Plant Research Lab, Michigan State University, MI

Plants vary greatly in their responses to cold temperatures. At one extreme are plants from tropical and subtropical regions such as soybean and rice, which suffer injury when exposed to chilling temperatures between 0 and 12°C. In sharp contrast, plants from temperate regions are not only chilling tolerant, but many, such as Arabidopsis and wheat, can survive freezing after exposure to low nonfreezing temperatures, a phenomenon known as "cold acclimation." Our long-range objective is to understand the genomic basis of this plant response. Much of our effort focuses on genes that are induced during cold acclimation. Recent studies with Arabidopsis have established that cold acclimation involves action of the CBF cold response pathway, a regulon of genes controlled by expression of the CBF transcriptional activators. Our current aim is to construct a low temperature "wiring diagram" of Arabidopsis that includes the identification of gene regulons and regulatory circuits that have important roles in cold tolerance. In addition, in a collaborative project funded by the NSF Plant Genome Research Program, we are determining whether the Arabidopsis CBF cold-response pathway is highly conserved in plants and whether differences in plant cold tolerance can be traced to differences in CBF cold-response pathways.

WHOLE-GENOME TRANSCRIPTIONAL ANALYSIS OF ARABIDOPSIS USING MASSIVELY-PARALLEL SIGNATURE SEQUENCING (MPSS)

Blake Meyers

Delaware Biotechnology Institute, University of Delaware, 15 Innovation Way, Newark, DE 19711, USA.

We have generated a collection of 36,991,173 17-base sequence tags or "signatures" representing transcripts from the model plant Arabidopsis. These data were derived by massively parallel signature sequencing (MPSS) from 14 libraries and comprised 268,132 distinct sequences. For each library, comparable data were obtained with 20-base signatures. We developed a method for handling these data and for comparing these signatures to the annotated Arabidopsis genome. As part of this procedure, 858,019 potential or "genomic" signatures were extracted from the Arabidopsis genome and classified based on the position and orientation of the signatures relative to annotated genes. A comparison of genomic and expressed signatures matched 67,724 signatures predicted to be derived from distinct transcripts and expressed at significant levels. Expressed signatures were assigned to 19,088 of 29,084 annotated genes. A comparison of the representation of four-base words in the genomic and expression signatures demonstrated that ~7.7% of genomic signatures were under-represented in the expression data. These signatures contained one of 20 four-base words in either MPSS sequencing frame that did not sequence well. More than 89% of the sum of the expressed signature abundances matched the Arabidopsis genome, and many of the unmatched signatures found in high abundances were predicted to match to previously uncharacterized transcripts. We have developed a publicly available database and interface with which to view the MPSS transcriptional data and the genomic locations for these signatures (http://mpss.udel.edu/at). These data are the first large-scale quantitative expression data for plants in the public domain.

THE USE OF DATA WAREHOUSING AND ONLINE ANALYTICAL PROCESSING (OLAP) TO IDENTIFY RESISTANCE GENES AND PATHWAYS IN A RESISTANT SOYBEAN CULTIVAR (PEKING) AGAINST THE CYST NEMATODE

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Changes in gene expression in roots of soybean, cv. Peking, resistant to attack by the soybean cyst nematode (SCN), *Heterodera glycines,* at 6, 12 and 24 hours, 2, 4, 6, and 8 days post invasion were monitored using microarrays containing over 6,000 cDNA inserts. Replicate, independent biological samples were examined at each time point. We used database warehousing and online analytical processing (OLAP) in conjunction with statistical database applications to analyze the gene expression data. OLAP was used as a data mining tool to quickly and efficiently find patterns and biological insights into the resistance pathways of soybean against SCN. OLAP was found to supplement cluster analysis and discover new candidate resistance genes that cluster analysis did not. The salicylic acid (SA) inducible genes PR-1a and NPR1 were found to be statistically induced using T-tests and OLAP. The induction of the SA defense pathway leads to the resistance against a wide range of pathogens and parasites in a number of plant species. Jasmonic acid (JA) inducible genes were also induced, these included lipoxygenase, chalcone synthase and PR-6. Other candidate resistance genes that were found induced were ubiquitin, glutathione s-transferase (GST) and aquaporin, all of which are involved in the systematic defense mechanism of plants against pathogen attack. Our results suggest that a combination of different defense related pathways, including the SA and JA inducible pathways, are joined together to tailor a response in Peking that confers its resistance to SCN.

MAIZE GENOME STRUCTURE, LINKAGE DISEQUILIBRIUM AND GENETIC ASSOCIATION MAPPING

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Genetic mapping of simple or complex (quantitative) traits is usually carried out in biparental mapping population developed specifically for this purpose. Recently, a lot of interest has been generated by an alternative approach, genetic association mapping. This approach explores the statistical relationship between the alleles or haplotypes present in pre-existing collections of germplasm and the traits of interest. This method may be applied to specific genes (candidate-gene based association mapping) and to the whole genome (whole genome scan). The choice between these alternatives depends on several factors, including the extent of linkage disequilibrium in the maize populations used for genetic association mapping. We show that the amount of LD is very dependent on the nature of the population used, and varies widely across the genome. Recently, DNA sequence level comparison of maize inbred lines at the bronze locus (H. Dooner and collaborators) demonstrated that two maize alleles could differ in their gene complement and in the content on non-genic insertions and deletions. We have now extended these studies to other loci. The presence of extensive allelic non-homologies has a significant impact on recombinational properties of the genome, and on the distribution of linkage disequilibrium in populations. The impact of these factors on genetic association mapping, and on breeding strategies, will be discussed.

HIGH THROUGHPUT SEQUENCING AND ANALYZING OF THE CDNAS OF HYPOTHETICAL GENES OF ARABIDOPSIS ON CHROMOSOME 2

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In fully sequenced Arabidopsis genome, about 20% of genes are called "hypothetical gene". This particular group of genes has structures that are predicted solely by computer algorithms with no support from either nucleic acid or protein homologs from other species or expressed sequence matches from Arabidopsis. A high throughput method was developed to clone and analyze cDNAs of these genes. Primers from 797 hypothetical genes on chromosome 2 were designed and clones from 5' or 3' RACEs of 506 genes were obtained and sent to sequence. The generated 11327 sequences match 399 genes from our target list. The cDNA sequences were obtained by sequencing and assembling 5' and 3' RACE products, which displays that the structures of 151 hypothetical genes are different with their predicted gene structures. In total, 87 hypothetical genes have alternatively spliced transcripts and 110 genes display more than one polyadenylation site. In addition, transcripts from opposite strands of their predictions, transcripts from both strands at same regions and dicistronic transcripts were found. The cDNA populations used in this study are from cold, heat, 2,4-D, H_2O_2, UV, IAA, salt-treated and pathogen (*Xanthomonas campestris* pv. campestris and *Pseudomonas syringae*)-infected plants, callus, roots and young seedlings. The promoter regions of 5 hypothetical genes were cloned into GFP and GUS reporter constructs and their expression patterns show tissue and development stage specific.

THE *MEDICAGO TRUNCATULA* GENOME SEQUENCING PROJECT: STRATEGY, STATUS AND RESULTS

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Legumes are one of the world's most important crop families, unique in their ability to fix atmospheric nitrogen. Among legumes, *M. truncatula* with a genome size of ~ 500 Mb is considered an excellent model for genome research. Unlike most crop legumes, *M. truncatula* has a compact genome, simple genetics, short generation time, relatively high transformation, excellent mutants, a large collection of ecotypes and an active research community. Comparative genomics demonstrates that the *M. truncatula* genome is highly syntenic with the genomes of alfalfa and pea and substantially conserved with several other legumes including soybean. Genomic resources already developed include a large collection of ESTs, several BAC libraries and an FPC-based physical map that is linked in many places to the genetic map. Because of these desirable features, *M. truncatula* is now the subject of an international sequencing effort. Persuasive evidence based on fluorescent in situ hybridization (FISH) of sequenced BACs to pachytene chromosomes demonstrates that the *M. truncatula* genome is organized into pericentromeric heterochromatin, rich in repeats, and separate gene-rich euchromatic chromosome arms. Thus, the overwhelming majority of the gene space, estimated to be 200-250 Mb, can be sequenced in a highly efficient manner using a BAC-based approach. The international sequencing consortium consists of groups from the USA (University of Minnesota, Oklahoma University and The Institute for Genomic Research) and the EU (INRA-Toulouse with Genoscope and the John Innes Centre with the Sanger Centre). Chromosomes 1, 4, 6 and 8 will be sequenced by OU, 2 and 7 by TIGR, 3 by JIC/Sanger and 5 by INRA/Genoscope. As of July 1, 2004 there was approximately 50 Mb of finished sequence and 50 Mb of "sequencing in progress" in GenBank. The project aims to complete the sequencing of the euchromatic gene-rich space by the end of 2006. During this sequencing phase of the project, automated bioinformatics pipelines are being used by several groups to provide working annotation of the sequence. The US project coordinating web site is at www.medicago.org and provides links out to the various web sites providing annotation and other information in both the USA and the EU. In this talk, I will describe the sequencing strategy and the current status of the project and highlight some of the features of the *M. truncatula* genome organization and gene content that have been revealed to date.
The US effort is supported by the National Science Foundation, and previous sequencing at OU was supported by the Noble Foundation.

Vernalization and the epigenetic memory of winter

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Certain plants, such as biennials or winter annuals, require relatively long periods of cold exposure to initiate flowering. Cold exposure renders the meristem of such cold-requiring species competent to flower, and this acquisition of competence is known as vernalization. A vernalization requirement ensures that flowering does not occur prematurely before the onset of winter. Molecular and genetic studies of vernalization in Arabidopsis have revealed that the state of expression of the gene FLOWERING LOCUS C (FLC) is a major component of meristem competence. FLC encodes a MADS-domain protein that acts as an inhibitor of flowering: high levels of FLC expression prevent the shoot apical meristem from initiating flowering. Exposure to prolonged cold causes an epigenetic switch of FLC to an unexpressed state and this repression of FLC renders the apical meristem competent to flower. I think it is reasonable to refer to this vernalization-mediated acquisition of the ability to flower as an epigenetic switch. This is a switch that is mitotically stable in the absence of the inducing signal; in this case, a stable acquisition of competence in meristem cells after cold is no longer present. The modifications of FLC chromatin that are involved in this epigenetic switch will be discussed.

DISSECTION OF GUARD CELL ABA SIGNAL TRANSDUCTION MECHANISMS USING COMBINED SINGLE CELL-TYPE FUNCTIONAL GENOMICS AND CELL BIOLOGICAL APPROACHES

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Guard cells have been developed for dissecting early signal transduction mechanisms. Relatively few signal transduction components have been identified from recessive ABA insensitive disruption mutants known to function during early ABA signal transduction upstream of transcription. The limited number of genetically identified positive ABA transducers is most likely due to redundancy in genes encoding ABA signaling components. To overcome this limitation and to dissect redundant signal transduction proteins, we have developed an alternative "single cell-type genomics" approach. This approach includes gene chip experiments performed with *Arabidopsis* guard cell RNA and degenerate oligo-based PCR screening of *Arabidopsis* guard cell cDNA libraries. Data obtained from detailed molecular genetic and cell biological analyses demonstrate that two NADPH oxidase catalytic subunit genes play central roles as positive signal transducers in guard cell ABA signal transduction. In addition, comprehensive analyses of microarray experiments with *Arabidopsis* guard cell and mesophyll cell RNA will be presented. From the microarray results, we identify a strongly ABA-induced protein phosphatase 2C gene in guard cells. A T-DNA disruption mutation in this gene confers ABA-hypersensitive regulation of stomatal closing and seed germination. The presented data provide a basis for cell-type specific genomic scale analyses of gene function.

EXPRESSION PATTERNS OF A NOVEL *ATCHX* GENE FAMILY HIGHLIGHT POTENTIAL ROLES IN OSMOTIC ADJUSTMENT AND K^+ HOMEOSTASIS IN POLLEN DEVELOPMENT

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A combined bioinformatic and experimental approach is being used to uncover the functions of a novel family of *CHX* (cation/H^+ exchanger) genes in plants using *Arabidopsis thaliana* as a model. The predicted protein (85-95 kDa) of 28 *AtCHX* genes after revision consists of an amino-terminal domain with 10-12 transmembrane spans (~440 residues), and a hydrophilic domain of ~360 residues at the carboxyl end which is proposed to have regulatory roles. The hydrophobic, but not the hydrophilic, domain of plant CHX is remarkably similar to monovalent cation/proton antiporter-2 (CPA2) proteins, especially yeast KHA1 and Synechocystis NhaS4. Reports of characterized fungal and prokaryotic CPA2 indicate they have various transport modes, including K^+/H^+ (KHA1), Na^+/H^+-K^+ (GerN) antiport, and ligand-gated ion channel (KefC). The expression pattern of *AtCHX* genes was determined by reverse-transcription polymerase-chain-reaction, promoter-driven GUS expression in transgenic plants, -and Affymetrix ATH1 Genome Arrays. Results show that 18 genes are specifically or preferentially expressed in the male gametophyte and 6 genes are highly expressed in sporophytic tissues. Microarray data revealed that several *AtCHX* genes were developmentally regulated during microgametogenesis. An exciting idea is that CHX proteins allow osmotic adjustment and K^+ homeostasis as mature pollen desiccates and then re-hydrates at germination. The multiplicity of *CHX*-like genes is conserved in higher plants, but is not found in animals. Only seventeen genes, *OsCHX01-OsCHX17*, were identified in *Oryza sativa* ssp. japonica, suggesting further diversification in Arabidopsis. These results reveal a novel *CHX* gene family in flowering plants with potential functions in pollen development, germination, and tube growth.

Laser capture microdissection (LCM) and expressed sequence tag analysis of the syncytium formed by the soybean cyst nematode *Heterodera glycines* in *Glycine max* (soybean)

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Laser capture microdissection (LCM) was used to isolate nematode feeding sites (syncytia) from susceptible soybean (*Glycine max* L. Merr. Kent) roots inoculated with the soybean cyst nematode (SCN), *Heterodera glycines* Ichinohe. RNA was extracted from the syncytia and a cDNA library was constructed for expressed sequence tag (EST), analyses. The ~700 ESTs fall into 174 contigs. Approximately ten percent of the contigs are homologous to wound/pathogen-induced or syncytium enhanced genes. Approximately 33% of the contigs have homology to genes involved in a variety of cellular processes in plants. Approximately 15% of the contigs are either predicted proteins or are ESTs found only in soybeans. Another 42% of the contigs have no significant identity with any sequence. These data demonstrate that LCM can be used to micro-dissect the developing syncytium, specifically providing tissue suitable for mRNA preparation. The mRNA extracted from the developing syncytium has been used to identify genes that exhibit expression within and around the syncytium. More broadly, LCM can be used to investigate plant-pathogen interactions where spatial domains are difficult to separate.

THE SPECIFIC INTERACTION BETWEEN MAIZE β-GLUCOSIDASES AND A CHIMERIC PROTEIN CONTAINING DIRIGENT AND LECTIN DOMAINS

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ß-Glucosidases (ß-D-glucoside glucohydrolase, EC 3.2.1.21) catalyze the hydrolysis of aryl and alkyl ß-D-glucosides as well as ß-linked oligosaccharides. In maize, two β-glucosidase isozymes (Glu1 and Glu2) have been characterized with respect to structure, including 3-D structure, and function. Certain maize lines were reported to lack β-glucosidase activity ("null") based on zymogram data. We have shown that these lines have enzyme activity, but the enzyme is not extractable from them because a 32 kD protein (referred to as BGAF or β-glucosidase aggregating factor hereafter) specifically interacts with Glu1 and Glu2 during extraction, rendering the enzyme insoluble or poorly soluble. BGAF is related to proteins induced by jasmonic acid, salicylic acid, insect feeding, and salt stress. It is a chimeric protein consisting of two distinct domains: the disease response (also known as "dirigent") domain and the jacalin-related lectin (JRL) domain. Like β-glucosidase, BGAF-related proteins appear to be ubiquitous in occurrence. Of the 142 JRLs in databases, 127 are from seed plants, 14 from mammals, and one from bacteria. In contrast, the dirigent domain seems to be restricted to seed plants because all 72 entries in the database are from plants. To understand the mechanism of the ß-glucosidase-BGAF interaction, we constructed chimeric enzymes by domain swapping between the maize ß-glucosidase isozymes Glu1 and Gu2, to which BGAF binds, and the sorghum ß-glucosidase (dhurrinase) isozyme Dhr1, to which BGAF does not bind. The results of binding assays with 12 different chimeric enzymes showed that an N-terminal region (E50-V145) and an extreme C-terminal region (F466-A512) together form the BGAF binding site on the enzyme surface. In addition, we purified BGAF, determined its N-terminal sequence, amplified the BGAF cDNA by RT-PCR, expressed it in *E. coli* and showed that it encodes a protein whose binding and immunological properties are identical to the native BGAF isolated from maize tissues. It is not known at this time whether the entire BGAF molecule or one of its two domains are involved in β-glucosidase recognition and binding. Our current research is addressing these issues as well as the structure-function issues related to both β-glucosidase and BGAF.

EXPRESSION PATTERN OF DISPENSABLE SR PROTEIN GENES IN ARABIDOPSIS THALIANA

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RNA processing in eukaryotes is a highly complex process requiring numerous steps and factors, and is emerging as a key step in the regulation of plant gene expression. SR proteins are a well-defined family of splicing factors identified by a conserved RNA-binding domain of the RRM type and carboxyl-terminal arginine-serine (RS) repeats. This family of proteins and its characteristic features are conserved across all eukaryotes and animal SR proteins have established roles in splicing regulation. Most multicellular eukaryotes have multiple SR protein genes (there are nine in humans and 20 in Arabidopsis) while unicellular eukaryotes such as yeast may have only a single SR protein gene. SR proteins are known facilitate splicing and to bind to pre-mRNA molecules via exon splicing enhancers (ESEs). While specific SR protein genes are dispensable in Arabidopsis, mice carrying mutations in a single SR protein gene show early embryonic lethality. In order to explore when and where individual SR proteins are expressed throughout development, we have begun to determine the expression patterns of individual SR proteins via GFP fusions in Arabidopsis thaliana. Thus far we have examined expression patterns of three Arabidopsis SR proteins (SR1, RSZp21, and RSp31) in multiple tissues including roots, leaves, and flowers. GFP fusion genes for the remaining 17 SR proteins are currently being constructed. Spatial and temporal expression patterns relative to specific tissues and general organs for the three proteins which we have been able to study in detail will be presented.

EXTRACELLULAR ATP SIGNALING INDUCES THE ACCUMULATION OF SUPEROXIDE VIA NADPH OXIDASES IN *ARABIDOPSIS THALIANA*

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Extracellular ATP, a well-characterized signal in mammalian cells, induces the production of reactive oxygen species in phagocytes by the catalytic action of NADPH oxidase. Consistent with it being a signaling agent also in plants, extracellular ATP induces the accumulation of superoxide in Arabidopsis leaves in a biphasic, dose-dependent manner between 1 μM and 100 μM ATP. Plants with disrupted AtrbohD and AtrbohF genes did not accumulate superoxide when treated with ATP, providing evidence that NADPH oxidases are responsible for the superoxide accumulation. Additionally, ATP induced increased levels of AtrbohD mRNA in a dose-dependent manner. Inhibitors of mammalian P2-type ATP receptors abolished ATP-induced superoxide production, suggesting that the ATP signal may be mediated through P2-like receptors in plants. Intermediate signaling roles for cytosolic Ca^{2+} and CaM are likely, because the Ca^{2+} channel blocker, $LaCl_3$, Ca^{2+} chelator, BAPTA, and CaM antagonist, W7, reduced ATP-induced superoxide accumulation. Extracellular ATP enhances the expression of genes that are induced by various stresses: PAL1, LOX2, and ACS6. ATP was measured at wound sites and found to be within the range to induce superoxide accumulation and gene expression changes. These data support a potential role for extracellular ATP as a signal especially in wound and stress responses.

PRELIMINARY STUDIES ON MICROARRAY ANALYSIS OF GENE EXPRESSION DURING COLD ACCLIMATION IN BLUEBERRY

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To monitor changes in gene expression associated with cold acclimation in the woody perennial blueberry (*Vaccinium* spp.), a genomics approach base on cDNA microarrays was undertaken using ~2400 ESTs generated from 2 cDNA libraries that were constructed using RNA isolated from cold acclimated floral buds (collected in mid winter conditions) and non-acclimated floral buds (collected in fall). cDNA inserts were amplified from the plasmids, purified and arrayed onto glass slides. RNA was isolated from floral buds collected from field grown plants of the relatively cold hardy cultivar 'Bluecrop' that received 0, 70, 400, 800 and 1200 chill hours and from greenhouse/cold room-grown plants that received 0, 500, and 1000 chill hours. Total RNA was used to make cDNAs, which were labeled with Alexa Flour 555 and Alexa Flour 647 dyes. Dye-swap labeling was carried out on two biological replicates. After hybridization, slides were scanned, data normalized using lowess print-tip method and ratios of the two signals estimated to determine relative levels of transcripts after different exposures to low temperature. T-tests and ANOVA will be used to determine statistically significant up and down-regulated genes with cold acclimation under field and cold room conditions.

Poster Abstracts

DETECTING GENES EXPRESSED AT DIFFERENT STAGES OF REPRODUCTIVE ARREST IN *BRASSICA OLERACEA*

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Cauliflower (*Brassica oleracea* var. *italica*) and broccoli (*Brassica oleracea* var. *botrytis*) differ mainly in the stage of the reproductive development at which arrest takes place. The curd phenotype in cauliflower corresponds to an inflorescence meristem. In broccoli the arrest occurs at a more advance stage, just before anthesis. Based on *Arabidopsis* studies, the first hypothesis for the difference in the phenotype between these two varieties was a difference in the *BoCAL* gene. Later, a new model, integrating *BoAP1* gene was proposed. These partially redundant genes, and several identified since, are present in multiple copies in *B. oleracea*. The main goal of this study is to identify additional genes that may play a role in the arrest of *B. oleracea.* Understanding their specific and redundant function in the arrest requires quantification of gene expression and evaluation of models of coordinated gene action. By altering temperature, total RNA was isolated from Green Harmony plants arrested at three developmental stages: inflorescence meristem (cauliflower), floral meristem (intermediate) and floral bud (broccoli). Primers designed in highly specific regions of the genes of interest (MADS-box genes *BoCAL, BoAP1, FUL*, and the non MADS-box genes LFY and *TFL1*) and their paralogs were used in conventional RT-PCR and SYBR Green Real Time RT-PCR. Results showed an increased in *LFY/TFL1* ratio towards flowering and differences in the expression levels of paralogous genes. While *AP1-c, FUL-b*, and *FUL-d* levels increased at the stage of inflorescence meristem; *AP1-a, FUL-a*, and *FUL-c* expression was higher at a more advanced stage when plants were arrested in flower primordia. These results revealed that temperature not only determines curd initiation but also influences the fate of meristem and that paralogous genes may play different roles in reproductive development.

CHARACTERIZATION OF R1-LIKE GENES FROM TWO POTATO DIFFERENTIALS RESISTANT TO PHYTOPHTHORA INFESTANS RACES 5 AND 9

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At least 11 genes conferring race-specific resistance to late blight in wild *Solanum demissum* species have been introgressed into commercial *S. tuberosum* cultivars. These genes appear to function in a typical gene-for-gene interaction manner as indicated by the development of a hypersensitive response after attack by a specific *P. infestans* race. Thus far, R1 is the only gene cloned and fully characterized. However, recent reports indicated that the resistance provided by R1 can now be overcome, on average, by more than 79% of race 1 field isolates. On the other hand, resistance of differentials carrying genes R5 and R9 was overcome much less frequently (19% and 22%, respectively). Fore this reason, we attempted to clone and characterize the R genes present in differentials showing resistance to races 5 and9 of *P. infestans*.

Using a PCR approach, 7 genomic clones of 4.1 kb in length were obtained from differential R5. From R9, 5 genomic clones of 4.1 kb and 4.3 kb were obtained with the same technique, using two different sets of primers. Southern blot hybridizations and sequencing experiments indicate that multiple alleles are present in each differential's genome. In the case of R9, it appears that the amplified alleles can be classified into two significantly divergent groups. Three cDNA clones, of approximately 3.9 kb in length, have been obtained from R9. Their respective ORF's encode putative proteins of 1240, 1259 and 1269 amino acids. A truncated fourth cDNA clone of 3.4 kb, which encodes a protein of 1104 amino acids, was also found. However it has not been possible to confirm the existence of this allele either through PCR or Reverse Transcription-PCR (RT-PCR) experiments. RACE assays revealed the presence of a 5' untranslated region of up to 86 nucleotides and a 3' untranslated region ranging between 320 and 427 nucleotides. Sequence comparisons between genomic and cDNA clones as well as PCR and RT-PCR experiments indicate that these genes contain several intron and suggest that either the expression of several copies of the gene or an alternative splicing mechanism could be responsible for the transcription of the multiple cDNA variants.

PLANT GENOMES CENTRAL

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Plant Genomes Central (PGC) is an integrated, Web-based portal for plant genomics data and tools at the National Center for Biotechnology Information (NCBI). It provides access to large-scale plant genomic and EST sequencing data, with links to corresponding taxonomic database. Organisms that have Large-scale sequencing projects and/or where genetic maps are available can be viewed through the MapViewer. It allows the user to view and search an organism's complete genome, display chromosome maps, and get progressively greater levels of detail, down to the sequence data, if available. Currently MapViewer is available for *Arabidopsis thaliana, Avena sativa, Hordeum vulgare, Oryza sativa, Triticum aestivum, Zea mays,,_Lycopersicon esculentum,* and *Glycine max.* PGC also offers multiple genome search page which allows the user to perform searches across mapped genomes. The plant genome BLAST databases are locally collected subsets of GenBank accessions which are associated with the loci of the available maps. The BLAST result includes the opportunity for Map Viewer to display the BLAST hits in the context of other chromosomal landmarks.

CHARACTERIZATIONS OF FAR-RED LIGHT SIGNALING MUTATION IN *ARABIDOPSIS THALIANA*

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We report the characterization of a semi-dominant mutation fin5-1 (far-red insensitive 5-1) of Arabidopsis, which was isolated from genetic screening of phytochrome A (phyA) signaling components. Plants with the fin5-1 mutation exhibited a long hypocotyl phenotype when grown under far-red (FR) light, but not under red light. Physiological analyses implied that FIN5 might be differentially involved in diverse responses that are regulated by phyA under continuous FR light. Anthocyanin accumulation, gravitropic response of hypocotyl growth, and FR light-preconditioned blocking of greening were also impaired in the fin5-1 mutant, whereas photoperiodic floral induction was not, if at all, significantly affected. Moreover, light-regulated expression of the CHS, PORA and PsbS genes was attenuated in fin5-1 mutant plants, while the light-induced expression of CAB was normal. The mutation exhibited semi-dominance regarding control of hypocotyl growth in FR light. We suggest that FIN5 defines a novel branch in the network of phyA signaling in Arabidopsis.

KINESIN AND SPERMATID DIFFERENTIATION IN MARSILEA VESTITA

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The microspore of the water fern *Marsilea vestita*, contains a single cell that undergoes a series of nine successive mitotic divisions to produce 32 spermatids, six jacket cells and one sterile prothallial cell. Immediately after the dry microspore is placed into water, significant movement within the cytoplasm precedes the first division; certain proteins and mRNAs aggregate into zones that later become the spermatogenous initials of the gametophyte. Our working hypothesis is that the asymmetric redistribution of proteins and mRNAs is driven by microtubule-based motility systems. Kinesin-driven movements along cytoplasmic microtubules appear to be involved in this process. Our goal is to study how kinesin affects development in particular stages of spermiogenesis. We developed RNAi strategies to target the degradation of specific mRNAs to arrest development in the gametophyte. These published studies show that the pattern of translation in the gametophyte is precisely ordered, which indicates that certain proteins are required at specific stages of development. In the current study, dsRNA probes were made from a cDNA encoding kinesin-like protein isolated from our gametophyte library. dsRNAs made from this cDNA were added to populations of developing gametophytes to assess the time and stage of development at which this transcript becomes limiting. Development is arrested prior to the completion of all nine division cycles. Treated cells were then labeled with anti-centrin and anti-bð-tubulin antibodies to determine the stage of arrest and the effect of a kinesin null phenocopy on the known distributions of centrin and bð-tubulin proteins. The patterns of developmental disruption induced by the absence of this kinesin-like protein are readily discernable in cells fixed at various times during development and precede the onset of de novo basal body formation, cell and nuclear shaping, and ciliogenesis. (Supported by NSF grant MCB 0234423 to SMW).

SEQUENCE AND MEMBRANE ARRAY ANALYSIS OF TWO SUBTRACTION CDNA LIBRARIES FOR NEMATODE INFECTED SOYBEAN ROOTS

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Soybean cyst nematode (SCN) is the most economically destructive pathogen of soybeans. Identification of gene expression that is up regulated during infection by SCN and also highly specific to the infection site will provide additional tools to engineer SCN resistance in soybean. We have prepared two subtraction libraries to enrich for SCN-induced genes expressed in the early and late stages of the 30-day lifecycle of SCN in soybean roots. In addition to subtraction with cDNA prepared from un-inoculated roots, cDNA from SCN eggs were also used for subtraction. Two thousand cDNA clones were selected from each library and stored in 96-well microtiter plates. From each of these libraries 384 cDNAs were sequenced from both directions and the cDNAs arrayed onto nitrocellulose membranes. The membrane arrays were then hybridized to radioactively labeled cDNAs prepared from total root RNA isolated at 4, 12, and 20 days post-SCN-inoculation (PSI) and RNA from 0, 4, 12, and 20 days incubation with no inoculation. The 384 cDNAs from the early subtraction library (1, 2, and 4 days PSI) do not include any SCN transcripts or host genes that were significantly up regulated at our conditions. However, 193 of the 384 cDNAs from the late subtraction library (8, 12, and 20 days PSI) have high sequence identity with nematode sequences and most were associated with GenBank SCN ESTs from maturing nematodes. Moreover, a large number of the soybean cDNAs that were up regulated by the SCN infection in the late library were associated with previously identified nodulation (nod) genes. After the library construction and initial hybridization experiments, a surface sterilization of SCN eggs prior to hatching was added to the nematode preparation protocol, which greatly reduced Nod gene expression and a few other inoculation-associated gene expression.

Poster Abstracts

CHARACTERIZATION OF AOX GENE EXPRESSION IN RESPONSE TO MESA AND MEJA PRE-TREATMENT AND LOW TEMPERATURE IN PINK TOMATOES (*LYCOPERSICON ESCULENTUM*)[§]

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Methyl salicylate (MeSA) vapor increased resistance against chilling injury (CI) in freshly harvested pink tomatoes (*Lycopersicon esculentum* L. cv. Beefsteak). The expression patterns of alternative oxidase (AOX) before and during the chilling period demonstrated that pre-treatment of tomato fruit with MeSA vapor increased the transcript levels of AOX. We used 4 EST tomato clones of AOX from the public database that belong to two distinctly related families, 1 and 2 defined in plants. Three clones were designated as LeAOX1a, 1b and 1c and the fourth clone as LeAOX2. Using RT-PCR, all three Family 1 clones were expressed in leaf and fruit tissues, but RNA transcript from LeAOX1a of AOX subfamily 1 was present in much greater abundance than 1b or 1c. The presence of longer AOX transcript detected by Northern analysis in cold-stored tomato fruit was confirmed to be the un-spliced pre-mRNA transcript of LeAOX1a and LeAOX1b clones, while intron splicing of LeAOX1c clone was not affected by cold storage. The intron splicing event in AOX pre-mRNAs did not relate to its abundance level. Transcript levels of key genes involved in RNA processing (splicing factors: 9G8-SR and SF2-SR, fibrillarin and DEAD box RNA helicase) were altered by changes in storage temperature. The inhibition of the AOX intron splicing event and its relationship with the change in expression of RNA processing enzymes in cold stored tomato fruit was discussed.

IDENTIFICATION AND CHARACTERIZATION OF A BATTERY OF WHEAT GENES REGULATED BY HESSIAN FLY LARVAL FEEDING

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Establishment of a feeding site beneath the leaf sheath near the crown of developing seedlings in compatible interactions of Hessian fly (*Mayetiola destructor*) and wheat (*Triticum aestivum* L. em Thell.) result in larval growth and development. Physiological changes induced by virulent Hessian fly larvae reduce yields dramatically. Resistance is the most efficient means of protecting wheat plants against this pest. To date, 32 genes conferring Hessian fly resistance have been identified, yet none have been cloned. Although the gene-for-gene model has been demonstrated genetically to fit the wheat/Hessian fly interaction, the molecular basis of this interaction has not been described. We identified, through GeneCalling, 73 genes regulated by virulent and avirulent Hessian fly feeding. Validation of differential expression by quantitative real time PCR of these 73 genes revealed seven genes consistently responding to Hessian fly feeding across experiments involving the wheat line Iris, which carries the *H9* resistance gene, infested with v*H9* (compatible) and biotype L (incompatible) flies. The expression profile of the seven genes in response to Hessian fly larval feeding was gathered from experiments from the whole crown tissue, from individual leaf sheaths and also from crown tissue of seedlings having 1 or 21-30 larvae feeding at the base of the plant. Blast analysis of the seven sequences to the NCBI translated data base showed the following putative functions: Lipid transfer protein, Agglutinin isolectin 2 precursor, DNA repair RAD 23, Glutathione-S-transferase, Connective tissue growth factor, Flavanone 3-hydroxylase and a Sorbitol transporter. Expression profiles were also generated following: non- and viruliferous aphids carrying compatible viruses (barley yellow dwarf virus, PAV strain and cereal dwarf virus, wounding, armyworm infestation, chemical treatment (ABA, MeJA and SA) and water stress. Near full length cDNAs were cloned for all seven genes by RACE PCR for bioinformatics analysis. Virus induced gene silencing (VIGS) experiments are in progress to confirm the contribution of this battery of genes, which will yield a better understanding of the molecular responses of wheat to the Hessian fly.

Poster Abstracts

LASER CAPTURE MICRODISSECTION ISOLATION OF SOYBEAN SYNCYTIAL CELLS FORMED BY THE SOYBEAN CYST NEMATODE

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The soybean cyst nematode (SCN) is the most devastating pest of soybean in the US, causing an estimated one billion dollars in damage each year. Kent roots susceptible to the soybean cyst nematode (SCN), race 3, were harvested 8 days after infection by SCN strain NL1-RHp. The roots were fixed, embedded in paraffin, sectioned and syncytial cells were harvested using laser capture microdissection. MRNA was isolated and used to construct a cDNA library. Several hundred clones were one-pass sequenced as expressed sequence tags (ESTs) and the sequences were compared with those in GenBank for identification. These results are being used to identify pathways, genes, and metabolites important to sustaining the syncytium and feeding the nematode. Promoters of these genes may be useful in expressing genes or gene fragments to confer resistance to nematodes and other pests. See our web site at http://bldg6.arsusda.gov/benlab/ for further information.

VERIFICATION OF GENETIC IDENTITY IN THEOBROMA CACAO GERMPLASM USING MICROSATELLITE MARKERS

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Theobroma cacao is cultivated extensively in the tropical rain forest as the source of cocoa butter and powder for the confectionery industry. Cocoa germplasm must be maintained as collections of live trees in the field because storage of the recalcitrant seeds is impossible. Incorrect labeling of accessions has been a problem in national or international collections growing in tropical areas of cocoa producing countries. Comprehensive assessment of genetic identity is essential to reduce error and redundancy in these collections, thus improving the accuracy and effectiveness of cocoa germplasm conservation and utilization. Microsatellite markers provide a powerful tool for identification of mislabeled cultivars and assessment of genetic diversity in these genebanks. In the present study, we evaluated the effectiveness of 15 microsatellite primers for individual identification using cocoa germplasm maintained in the International Cocoa Genebank in Trinidad. The polymorphic information content (PIC) ranged from 0.42 to 0.82 with a mean of 0.67 among the 15 loci. The observed heterozygosity ranged from 0.51 to 0.79. The accumulated probability of identity (PI) of the 15 loci varied from 7.87 x 10-5 and 1.485 x 10-9, depending on the population's genetic background. This result showed that the probability of a chance match between any two genotypes is negligible. Therefore, the combination of the 15 SSR primers is sufficient for cocoa individual identification. Using these 15 primers, we assessed the extent of mislabeling in 3 reference populations collected from the Amazon rain forest in the 1940s. Our result showed that only 2-3% of the original reference trees were mislabeled. Moreover, we were able to reconcile each of the mislabeled trees by comparing the SSR profile of the mislabeled one with its neighboring trees in the field.

SSR markers proved to be efficient and reliable for the molecular characterization of cocoa germplasm.

FAMILY CLASSIFICATION AND INTEGRATIVE ANALYSIS OF MOLYBDENUM COFACTOR BIOSYNTHESIS PROTEINS AND RELATED PROTEINS

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PIRSF is a network protein family classification system that reflects evolutionary relationships of full-length proteins. The primary PIRSF classification unit is the *homeomorphic family* whose members are both *homologous* (evolved from a common ancestor) and *homeomorphic* (sharing full-length sequence similarity and a common domain architecture). Here, we present family classification and detailed annotation of some of the molybdenum cofactor (Moco) biosynthesis proteins. PIRSF classification system allows annotation of specific biological as well as generic biochemical functions, and sequence features that are family (or subfamily) specific.
Moco is the essential component of a diverse range of redox-active enzymes. The cofactor consists of a mononuclear molybdenum coordinated by the dithiolene moeity of tricyclic molybdopterin (MPT). Genes involved in Moco biosynthesis have been identified in eubacteria, archaea and eukaryotes. Moco biosynthesis pathway can be divided into three universal stages:

1) Precursor Z biosynthesis (e.g., familiesPIRSF003315 and PIRSF004845),
2) MPT biosynthesis (e.g., families PIRSF006462, and PIRSF005983),
3) Moco biosynthesis with molybdenum incorporation (e.g., families PIRSF036627, PIRSF036598 with subfamily PIRSF500055),
4) An addditional step of Moco dinucleotide biosynthesis (e.g., families PIRSF036607 and PIRSF036622) is also required in eubacteria.

Domain shuffling is observed in Moco biosynthesis-related proteins families, and functions of some families are predicted based on their domain architectures. Protein families sharing domains with Moco biosynthesis protein also have been investigated. Interestingly, MPT binding domain PF00994 shared by most Moco molybdenum incorporation proteins is also present in competence/damage-inducible protein CinA (PIRSF006728) and some eukaryotic FAD synthetases (PIRSF036620).

Curated Moco biosynthesis protein families illustrate how various types of data from diverse sources are integrated in the PIRSF system to allow the user a faster and more accurate analysis of protein sequence and function. Such integrative analysis includes position-specific site rules (active site residues annotation), 3D structure information, genome context, phylogenetic comparison, etc.

Data integration and analysis allows standardizing nomenclature and gaining insights into the evolution of Moco biosynthesis proteins and of the corresponding pathway.

FUNGAL RESPONSE TO PLANT DEFENSE COMPOUNDS: A TWO-HYBRID APPROACH TO DISSECT THE SIGNAL TRANSDUCTION 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 (Pisatin Response Factor) 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. Binding studies in bacteria, *Escherichia coli* and yeast, *Saccharomyces cerevisiae* showed a decreased binding of PRF to DNA in response to pisatin. But similar studies in *F. solani* and related fungus, *Neurospora crassa* showed an increased binding. This discrepancy in the binding results suggests the presence of a heterodimeric partner to PRF absent in simpler heterologous systems. The two-hybrid analysis has revealed two promising proteins showing high levels of interaction with PRF – V25 and V27. V25 is a novel protein, similar to the Mn/Fe superoxide dismutase but lacking all the expected domains. The protein is predicted to possess a mitochondrial targeting sequence and a nuclear localization signal. Predominantly predicted to be localized in the mitochondria, this protein is also likely to be present in the nucleus. V27 was found to have a nuclear localization signal. Currently, binding studies are underway to establish an *in vitro* interaction between V25 and PRF. 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.

MOLECULAR IDENTIFICATION OF THE 'BLACK SHADOW' PATHOGEN OF BLUEBERRY

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'Black Shadow' is an emerging disease on highbush blueberries grown in New Jersey. The disease appears as black mycelium on the surface of 1-2 year old stems. Affected bushes generally decline and have reduced yields. In culture, isolates of the fungus grow yeast-like, as single cells that proliferate by budding. Observations based on morphology and growth habit led to preliminary identification of the causal agent as *Aureobasidium pullulans*. This conclusion was supported by an abstract published several years ago describing *A. pullulans* as pathogenic on blueberry. To verify identification, the nuclear rDNA region containing ITS2, 5.8S rDNA and ITS 4 was amplified from several isolates of the fungus, cloned and sequenced. Sequence similarity searches using BLAST suggested the isolated species was *Rhizosphaera kalkoffii*. Interestingly, this species of fungus has only been reported as a pathogen of conifers where it causes a disease known as 'needle cast'.

Poster Abstracts

CALCIUM ATPase ATECA3 SHOWS TISSUE-SPECIFIC EXPRESSION AND SUPPORTS ROOT AND POLLEN TUBE GROWTH

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In plant cells, several Ca-ATPase homologues are proposed to maintain sub-micromolar [Ca^{2+}]cyt and to load mM [Ca] into endomembrane compartments, though the specific roles of many ACA and ECA gene products from Arabidopsis are unknown. AtECA1, an ER-bound Ca/Mn pump, supported plant growth and conferred tolerance to Mn^{2+} stress (Wu et al. 2002, Plant Physiol 130:128). We are now investigating the biological roles of 3 other AtECA. AtECA3 (At1g10130) is strikingly different, as it shares higher identity (54%) with human ER/SER Ca^{2+} pumps than with three other AtECA (47%). AtECA3 expression in tissues as monitored by promoter::Gus analyses was extremely low compared with ECA1, ECA2 or ECA4. Expression of ECA3 was detected only in vascular tissues of root, young cotyledons and leaves. Here we report that gene disruptions in ECA3 result in at least two observable phenotypes, as corroborated with three independent alleles (eca3-1, eca3-4 and eca3-5). First, mutant pollen showed a 39% decrease in growth when assayed for in vitro pollen tube growth potential. Second, root growth was reduced in mutants (~35%) when grown on medium containing low or high levels of Ca^{2+}. While this root growth phenotype has similarities to that observed with another ECA mutant (eca1-1) (Wu et al. 2002. Plant Physiol. 130, 128), the expression level of ECA3 based on RNA gel blot and on promoter::GUS analyses is very low and tissue-specific compared to ECA1. The pollen phenotype for eca3 mutants provides the first evidence for the role of an ECA in pollen growth. The results suggest that ECA3 supports biological functions distinct from other ECAs. How the biochemical activity, cell-type and sub-cellular location of ECA3 differ from ECA1, ECA2, and ECA4 have yet to be determined. (Supported in part by DOE grants to HS and JFH).

INSECT HERBIVORY AND VOLICITIN INDUCE GENES INVOLVED IN VOLATILE PRODUCTION

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In crop plants, mechanical damage and insect attack trigger rapid changes in gene transcription. Does insect herbivory differ from a general wound response? If so, is this induction specific to the pest/host plant interaction? Herbivory by beet armyworm (BAW) caterpillars on maize results in a unique pattern of volatile compounds not triggered by wounding alone. This bouquet of volatiles attracts the generalist parasitoid, *Cotesia marginiventris* and enables these natural enemies to locate the offending caterpillars. Caterpillar induced volatile emission can be mimicked when volicitin, a component of the BAW regurgitant, is added to a wounded leaf.

We have identified genes that are affected by BAW feeding by comparing volicitin treatment with wounding alone. We compared cDNAs from these two populations by isolating genes from a subtractive library and using reverse northerns. Virtual northern blots confirmed these results and further showed that BAW infestation affected the expression of these genes. In some cases, BAW feeding inhibited the expression of volicitin-induced genes suggesting the role of additional bioactive components in caterpillar regurgitate. Transcripts involved in volatile production are increased by volicitin and BAW infestation treatments, and are also detectable at low levels in mechanically wounded leaves. Finally, we identified three new sesquiterpene cyclase genes that are induced by volicitin and may be involved in the biosynthesis of volatile compounds.

COMPARATIVE TRANSGENE EXPRESSION IN 'CHARDONNAY' BETWEEN MINIMAL CASSETTE AND CIRCULAR PLASMID TRANSFORMATION

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Minimal gene cassette (MC) was compared to traditional whole circular plasmid (CP) for transformation of grapevine (*Vitis vinifera* L.). Embryogenic cell suspensions of 'Chardonnay' were transformed via particle co-bombardment using two non-linked genes into either CP or MC, which is a linear DNA fragment (promoter-open reading frame-terminator) lacking the vector backbone sequence. One construct contained the *npt-II* selectable marker gene and the second construct contained the *MSI99* antimicrobial peptide gene, which belongs to the magainin family. Both genes were driven by *Arabidopsis* ubiquitin promoters. A total of 5 and 10 independent lines from MC and CP, respectively, showed positive signals by PCR for both *npt-II* and *MSI99* gene. Hybridization signals from Southern blot analyses revealed 1 to 3 integration events in both DNA treatments. Transcription levels determined by quantitative RT-PCR varied among transgenic lines. Significant differences in transgene expression between lines from MC and CP DNA treatment were not found. The correlation between npt-II and MSI99 transcription level was positive with significant relationship ($P<0.05$), however no correlation between transcription level and integration events were observed. Regenerated greenhouse-grown plants were inoculated and evaluated for resistance to *Agrobacterium vitis*, the causal agent of crown gall disease. Initial tests indicated that bacteria population was lower in 2 transgenic lines compared with non-transgenic lines. Most of transgenic lines presented a normal phenotype in leaf morphology and common plant vigor compared to non-transgenic lines. Moreover, transgenic lines from both MC and CP DNA treatments produced grape clusters as did the non-transgenic lines in the third year growing in the greenhouse. Our preliminary data confirm the effectiveness of the minimal cassette "clean gene" technology for genetic transformation of elite grapevine cultivars to avoid potential negative effects on transgene and endogenous gene expression associated with the integration of plasmid backbone sequences.

HIGH THROUGHPUT SEQUENCING AND ANALYZING OF THE CDNAS OF HYPOTHETICAL GENES OF ARABIDOPSIS ON CHROMOSOME 2.

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In fully sequenced Arabidopsis genome, about 20% of genes are called "hypothetical gene". This particular group of genes has structures that are predicted solely by computer algorithms with no support from either nucleic acid or protein homologs from other species or expressed sequence matches from Arabidopsis. A high throughput method was developed to clone and analyze cDNAs of these genes. Primers from 797 hypothetical genes on chromosome 2 were designed and clones from 5' or 3' RACEs of 506 genes were obtained and sent to sequence. The generated 11327 sequences match 399 genes from our target list. The cDNA sequences were obtained by sequencing and assembling 5' and 3' RACE products, which displays that the structures of 151 hypothetical genes are different with their predicted gene structures. In total, 87 hypothetical genes have alternatively spliced transcripts and 110 genes display more than one polyadenylation site. In addition, transcripts from opposite strands of their predictions, transcripts from both strands at same regions and dicistronic transcripts were found. The cDNA populations used in this study are from cold, heat, 2,4-D, H_2O_2, UV, IAA, salt-treated and pathogen (*Xanthomonas campestris* pv. campestris and *Pseudomonas syringae*)-infected plants, callus, roots and young seedlings. The promoter regions of 5 hypothetical genes were cloned into GFP and GUS reporter constructs and their expression patterns show tissue and development stage specific.

CLONING AND FUNCTIONAL EXPRESSION OF VICIANIN HYDROLASE, A CYANOGENIC GLYCOSIDASE, FROM *Vicia angustifolia*

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The cyanogenic diglycoside vicianin [mandelonitrile β-vicianoside (6-*O*-α-L-arabinopyranosyl-β-D-glucopyranoside)] accumulates mainly in seeds of *V. angustifolia*. Vicianin hydrolase (VH) hydrolyzes vicianin into mandelonitrile and a disaccharide, vicianose. The enzyme has been known to be involved in cyanogenesis, the production of HCN by further hydrolysis of mandelonitrile. VH was purified from seeds using DEAE-, CM-, and Con A-Sepharose chromatography. The molecular weight of the purified VH was 56 kD based on SDS-PAGE analysis. The N-terminal sequence of the purified VH was determined and found to be GTGTPSQEVHPSHY. A cDNA encoding VH was obtained by screening a cDNA library. The deduced sequence of the VH protein has 512 amino acids including a putative secretion signal peptide. The predicted VH protein shares about 50% sequence identity with various plant β-glucosidases including tea leaf β-primeverosidase and furcatin hydrolase, and it belongs to family 1-glycosyl hydrolases. The *VH* transcript was detected in seeds and flower, but not in leaf, stem and root, suggesting that organ distribution of the *VH* expression is similar to that of the substrate vicianin. The native VH as well as the recombinant VH expressed in insect cells with a baculovirus system hydrolyzed vicianin to release vicianose, demonstrating that VH is a disaccharide-specific β-glycosidase. VH also hydrolyzed β-primeverosides and β-acuminosides but did not hydrolyze β-gentiobiosides. VH showed activity on prunasin which contains the same aglycone as vicianin, but little activity toward 2-phenylethyl, *p*NP, and *p*-allylphenyl β-D-glucopyranoside. Taken together, VH exhibits substrate specificity for the aglycone moiety as well as the glycone moiety of the substrate.

EXPRESSION PATTERNS OF A NOVEL *ATCHX* GENE FAMILY HIGHLIGHT POTENTIAL ROLES IN OSMOTIC ADJUSTMENT AND K^+ HOMEOSTASIS IN POLLEN DEVELOPMENT

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A combined bioinformatic and experimental approach is being used to uncover the functions of a novel family of *CHX* (cation/H^+ exchanger) genes in plants using *Arabidopsis thaliana* as a model. The predicted protein (85-95 kDa) of 28 *AtCHX* genes after revision consists of an amino-terminal domain with 10-12 transmembrane spans (~440 residues), and a hydrophilic domain of ~360 residues at the carboxyl end which is proposed to have regulatory roles. The hydrophobic, but not the hydrophilic, domain of plant CHX is remarkably similar to monovalent cation/proton antiporter-2 (CPA2) proteins, especially yeast KHA1 and Synechocystis NhaS4. Reports of characterized fungal and prokaryotic CPA2 indicate they have various transport modes, including K^+/H^+ (KHA1), Na^+/H^+-K^+ (GerN) antiport, and ligand-gated ion channel (KefC). The expression pattern of *AtCHX* genes was determined by reverse-transcription polymerase-chain-reaction, promoter-driven GUS expression in transgenic plants, -and Affymetrix ATH1 Genome Arrays. Results show that 18 genes are specifically or preferentially expressed in the male gametophyte and 6 genes are highly expressed in sporophytic tissues. Microarray data revealed that several *AtCHX* genes were developmentally regulated during microgametogenesis. An exciting idea is that CHX proteins allow osmotic adjustment and K^+ homeostasis as mature pollen desiccates and then re-hydrates at germination. The multiplicity of *CHX*-like genes is conserved in higher plants, but is not found in animals. Only seventeen genes, *OsCHX01-OsCHX17,* were identified in *Oryza sativa* ssp. japonica, suggesting further diversification in Arabidopsis. These results reveal a novel *CHX* gene family in flowering plants with potential functions in pollen development, germination, and tube growth.

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