Nineteenth Annual
Meeting **2002**

Mid-Atlantic Plant Molecular Biology Society

Beltsville, MD
August 19 & 20, 2002

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INTRODUCTION

Welcome to MAPMBS 2002, the nineteenth annual meeting of the Mid-Atlantic Plant Molecular Biology Society. The goal of this society and these meetings is to provide a forum for area scientists to exchange ideas and information relating to plant molecular biology. These meetings are designed to bring some of the best scientific minds to our area and to introduce some of the most interesting advances in plant molecular biology to our area scientists at a reasonable price and at an accessible location. We hope to entice a large number of students, postdocs and senior scientists to attend and actively participate in presentations and discussions. In addition, the meeting is designed to encourage mixing of scientists in an informal atmosphere during on-site lunches and breaks to provide each participant the opportunity to meet invited speakers and other members.

The meeting encompasses a large number of important 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 committees if you have thoughts or comments for consideration in the planning of future meetings; or join next years organizing team and volunteer your services to improve upon what we did this year. All are welcome at every stage of planning and organizing each meeting.

Many people were involved in the organization and planning of the meeting, and we give them our hearty thanks. We wish to thank our sponsors and exhibitors who furnish us with up-to-date product advances, and help to defray costs. Please visit our sponsors' and exhibitors' displays which are located with the posters. The level of interest you show in their products is a critical factor in their willingness to support future meetings.

We thank you for your continued support of and participation in the MidAtlantic Plant Molecular Biology Society. Enjoy the meeting!

Ben Matthews
Chair

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ABSTRACTS BOOK

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Rana Khan

PROJECTIONISTS

Liz Matthews
Emily Leamy

2002 MAPMBS MEETING SCHEDULE

Monday August 19

9:00 am Registration and Poster set-up

9:25 am Introductions

Session I – **Plant Pathology**
Moderator: Beth Grabau

9:30 am Rana Khan, USDA-ARS, Beltsville, MD
"Changes in gene expression profile of a resistant and susceptible soybean cultivar over time following soybean cyst nematode invasion"

9:50 am Steven Pechous, USDA-ARS, Beltsville, MD
"The role of HMG-CoA Reductase isoforms in the development of superficial scald in apple (*Malus domestica*) peel"

10:10am Jane J Choi, Department of Plant pathology, Washington State University,Pullman,WA
"Analysis of a pea defense gene promoter via agroinfiltration and its fusion with an elicitor-coding gene to develop non-host resistance"

10:30 am Corine M. van der Weele, Department of Cell Biology and Molecular Genetics University of Maryland, College Park, MD
"High resolution analysis of expansion and cell production dynamics underlying growth rate regulation in roots of Arabidopsis thaliana"

10:50 am **Break - Coffee, Poster Set-up and Viewing, Exhibitors (Bldg 003, Rm 20)**

11:30 am Vicky Vance, Biology Dept. University of South Carolina
"Viral suppression of RNA silencing in Plants"

12:00 noon Bruce Cahoon, Boyce Thompson Institute, Ithaca, NY
"Maize BMS cultured cell lines survive with massive plastid gene loss"

12:20 pm **Lunch (Bldg 005), Exhibitors and Poster Viewing (Bldg 003, Rm 20)**

Session II – **Bioinformatics & Genomics**
Moderator: John Hammond

1:20 pm Cathy Wu, Georgetown University Medical Center & Director of Bioinformatics, National Biomedical Research Foundation, MD
"Protein information resource for functional genomics and proteomics"

1:50 pm F. James Rohlf, Dept. Ecology and Evolution, SUNY Stonybrook, NY
"Searching for structure in multivariate data"

2:20 pm Rey Loor, National Cacao and Coffee Program, Quevedo, Ecuador
"Characterization of Ecuadorian cacao (*Theobroma cacao* L.)

2:40 pm M.L.Posada and R.D.Frederick, USDA-ARS Foreign Disease Weed Science Research Unit, Fort Detrick, MD
"First Assessment of gene expression in the soybean rust *Phakopsora pachyrhizi*"

3:00 pm **Break - Sodas, Exhibitors and Poster Viewing**

Keynote Address
Introduction: Ben Matthews

3:45 pm Daniel Klessig, Boyce Thompson Institute for Plant Research
"SA - and NO - mediated signal transduction in plant disease resistance"

5:00 pm Close of day

Tuesday August 20

Session I – **Plant Development**
Moderator Frank Turano

9:15 am Steve Wolniak (University of Maryland, College Park)
"Patterns of translation that lead to rapid development during spermiogenesis in *Marsilea vestita*"

9:45 am Janet Slovin, Fruit Laboratory, USDA-ARS, Beltsville, MD
"J-domain proteins in tomato and strawberry that are heat shock proteins and are expressed in reproductive tissues"

10:05 am Kevin Forbes, University of Kentucky, Lexington, KY
"Characterization of an Arabidopsis gene encoding a Fip1 homolog involved in polyadenylation"

10:25 am **Break - Coffee, Exhibitors and Poster Viewing**

Session II – **Plant Engineering**
Moderator: Frank Turano

11:00 am	Craig Nessler, Dept. of Plant Pathology, Physiology and Weed Science, Virginia Tech. "Plant metabolic engineering: From the pharmacy to the farm"
11:30 am	Carole Cramer, Dept. of Plant Pathology, Physiology and Weed Science, Virginia Tech. "Mucosal immunity and the mean green vaccine"
12:00 pm	**Business Meeting**
12:10 pm	**Lunch (Bldg 005) Exhibitors and Poster Viewing (Bldg 003)**

Session III – **RNA regulation and Mapping**
Moderator: Janet Slovin

1:30 pm	Pam Green, Delaware Biotechnology Institute, Newark, DE "Rapid mRNA decay mechanisms: Insight from genetic and genomic approaches"
2:00 pm	Gustavo MacIntosh, Delaware Biotechnology Institute, Newark, DE "Extracellular ribonuclease activity negatively affects growth of Arabidopsis plants"
2:20pm	Palaisa K.A, University of Delaware & Delaware Biotechnology Institute, Newark "Sequence diversity and long range linkage disequilibrium around the maize Y1 locus"
2:40pm	**Closing Remarks**
	Poster Take-down

2001 MAPMBS Poster sessions

Poster	Page	
1	29	**An Michiels**, Dominik Van Wonterghem, Wim Van den Ende, Rudy Vergauwen and André Van Laere, Dept. of Biology, Botany Institute, KULeuven, Kasteelpark Arenberg, 31, B-3001 Heverlee, Belgium. **Transcriptional regulation, tissue-specific and developmental expression of 1-SST (sucrose: sucrose 1-fructosyl transferase) from *Taraxacum officinale***
2	30	**J.L. Hampton**, D.M. Livingstone, T. Boluarte-Medina, F. Medina-Bolivar, B.B. Shew, J. Hollowell, P.M. Phipps, E.A. Grabau. Department of Plant Pathology, Physiology and Weed Science, Virginia Tech, Blacksburg, VA 24061, Tidewater Agricultural Research & Extension Center, Suffolk, VA 23437 and Dept. of Plant Pathology, North Carolina State University, Raleigh, NC 27695. **Growth and oxalic acid production in liquid cultures by isolates of *Sclerotinia minor***
3	31	**Keli K. Agama**, Lynn K. Carta, Susan L.F. Meyer and Andrea Skantar, USDA-ARS Nematology Laboratory, PSI, Beltsville, MD 20705. **Molecular analysis of Hsp90, a multifaceted gene involved in the growth and development of free-living and plant parasitic nematodes.**
4	32	**Snezana D. Ivic** and Ann C. Smigocki. USDA-ARS, Molecular Plant Pathology Laboratory, Beltsville, MD. **Biolistic transformation of sugar beet cell suspensions**.
5	33	**Nadim A. Alkharouf** and Benjamin Matthews. USDA-ARS, Soybean Genomics and Improvement Laboratory, Beltsville, MD **Recent updates to the soybean genomics and microarray database**
6	34	**S. Mehra,** A. Dhanaraj, G.R. Panta, C. Parmetier-Line, and L.J. Rowland, USDA-ARS, Fruit Laboratory, Beltsville, MD. **Use of molecular genetic and genomic approaches for the study of cold hardiness in blueberry**
7	35	**Senthilkumar Padmanaban**, Xiaoying Lin, and Heven Sze Dept. of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD **Differential expression and roles of the vacuolar H^+-ATPase subunit c1 and c3 in plant growth**
8	36	Carole L. Bassett, Timothy S. Artlip and Michael E. Wisniewski USDA-ARS, Appalachian Fruit Research Station, 45 Wiltshire Road, Kearneysville, WV

Seasonal differences in transcript initiation from promoters of two peach dehydrin genes

9 37 Preetmoninder Lidder[1], Miguel A. Perez-Amador[2] and Pamela J. Green[1]
[1]Delaware Biotechnology Institute, Newark DE, [2]Instituto de Biologia Molecular y Cellular de Plantas, Universidad de Valencia, Spain
Characterization of the DST-mediated decay pathway in *Arabidopsis thaliana* utilizing genetic techniques in conjunction with microarray technology

10 38 Sharmila Mallya and Asim Esen Virginia Polytechnic and State University, Blacksburg, VA
Oligomerization of β-glucosidases in maize and sorghum

11 39 C. He, V. Poysa and K. Yu Soybean Genomics and Improvement Laboratory, Beltsville Agricultural Research Center, Beltsville, MD 20705
Genetic analysis and SSR tagging of the gene conditioning powdery mildew resistance in the tomato hybrid DRW4409

12 40 Raul F. Medina Pedro Barbosa and James A. Saunders
Beltsville Agricultural Research Center, Beltsville, MD 20705
THE ROLE OF HOST-PLANT ASSOCIATION ON THE GENETIC DIFFERENTIATION OF SYMPATRIC POPULATIONS OF *Aleiodes nolophanae* (Ashmed) (Hymenoptera: Braconidae)

CHANGES OVER TIME IN GENE EXPRESSION PROFILE OF A RESISTANT AND SUSCEPTIBLE SOYBEAN CULTIVAR FOLLOWING SOYBEAN CYST NEMATODE INVASION

Rana Khan, Benjamin Matthews, Nadim Alkharouf, Imed Chouikha, Hunter Beard, Margaret MacDonald
Soybean genomics and Improvement Laboratory, USDA-ARS, Beltsville, MD

The soybean cyst nematode (SCN) is the major pest of soybean in the US and causes an estimated one billion dollars worth in damage each year. The defense response of soybean to SCN is a multigenic trait and varies depending upon the genotypes of soybean and SCN. The expression of over 3000 soybean genes was monitored using microarrays to identify genes involved in the response of soybean to SCN. RNA was harvested from roots of soybean cv. Peking resistant to SCN race 3 and cv. Kent susceptible to SCN race 3, either not infected or at several time points after infection by SCN race 3. The isolated RNA from each time point was fluorescently labeled as cDNA for hybridization to the microarrays. A number of defense related genes were at least 2-fold induced in the presence of SCN in both the cultivars. Besides the defense-related genes other induced genes included potential regulatory factors such as phosphatases and transcription factors, genes involved in sugar metabolism and cell wall formation, and a number of genes encoding proteins of unknown function.

THE ROLE OF HMG-COA REDUCTASE ISOFORMS IN THE DEVELOPMENT OF SUPERFICIAL SCALD IN APPLE (*Malus domestica*) PEEL

STEVEN W. PECHOUS and Bruce D. Whitaker – USDA-ARS Plant Sciences Institute, Produce Quality and Safety Laboratory, Beltsville, MD 20705

As part of an effort to elucidate the mechanism of superficial scald in apple fruit, and to devise a molecular genetic strategy for control of this storage disorder, we have begun to clone and characterize gene products that regulate production of a-farnesene in peel tissue. Oxidation products of this sesquiterpene are thought to induce necrosis of hypodermal layers just beneath the fruit skin, leading to development of scald symptoms. Here we report the cloning and bacterial expression of a cDNA encoding 3-hydroxy-3-methylglutaryl CoA reductase (HMGR), the initial, rate-limiting enzyme in sesquiterpene biosynthesis via the mevalonic acid pathway. RT-PCR cloning based on the conserved catalytic domains of plant HMGRs yielded three different cDNA fragments from peel tissue mRNA, designated *HMG1*, *HMG2*, and *HMG3*. A full-length transcript of *HMG1* (2334 bp) was obtained from an apple peel cDNA library, with an open reading frame of 1827 bp, encoding a protein 608 amino acids in length. A partial *HMG3* clone was isolated (1190 bp), composed of a 1140-bp coding region and a 50-bp 3' untranslated region. We have also obtained another partial *HMG2* gene, a short fragment of which was previously isolated in another lab. RNA-gel blots of *HMG1* showed a high level of expression in peel tissue at harvest and after 4 and 8 weeks of storage at 0.5 °C, even when tissue responsiveness to ethylene was blocked by prestorage treatment of fruit with 1-methylcyclopropene. Hence, the rapid rise in a-farnesene production that occurs in the initial weeks of storage cannot be attributed to increased expression of *HMG1*. E. coli cells transformed with the *HMG1* transcript showed over 10-fold higher HMGR activity than those with the control vector. Bacterial expression was confirmed by immunoblots of HMG1 protein fused to a C-terminal *myc* tag, which showed a single band with a molecular mass of ~ 69 kDa. Further data concerning the expression of *HMG2* and *HMG3* under similar (ethylene action-inhibiting) conditions will be presented.

ANALYSIS OF A PEA DEFENSE GENE PROMOTER VIA AGROINFILTRATION AND ITS FUSION WITH AN ELICITOR-CODING GENE TO DEVELOP NON-HOST RESISTANCE

Jane J. Choi and Lee A. Hadwiger, Department of Plant Pathology
Washington State University, Pullman, WA 99164-6430

Plant non-host disease resistance is characterized in part by the induction of multiple defense genes, which act as deterrents against diverse and potentially harmful organisms. The pea DRR206 gene is induced following inoculation with pathogens, treatment with abiotic agents, and to a moderate extent by wounding. In this study, a deletion series of DRR206 promoter segments were fused with the GUS reporter gene and transiently transferred to tobacco, potato, and pea. Analyses of GUS activity in leaf tissues from these different plants revealed that two upstream regions of the DRR206 promoter were particularly important for activation in the three plant species. Several database search tools revealed the presence of putative *cis* regulatory elements within the DRR206 promoter, including a wound/pathogen inducible box (W/P-box) and a WRKY box (W-box). Gel shift assays with nuclear extracts from treated and untreated tissue with the W/P-box revealed both similar and unique protein-DNA complexes from pea, potato, and tobacco. Stable transformations of tobacco were performed with gene constructs of the DRR206 promoter fused with a DNase elicitor gene from *Fusarium solani* f. sp. *phaseoli*, FsphDNase. Pathogenicity tests indicated that the FsphDNase elicitor conferred resistance against *Pseudomonas syringae* pv *tabaci* and *Alternaria alternata* in tobacco and in potato showed resistance against *Phytophthora infestans*. These studies demonstrate that the elicitor-coding gene, FsphDNase, is capable of inciting pathogen resistance in a heterologous plant system when fused with defined regions of the pea DRR206 promoter.

HIGH RESOLUTION ANALYSIS OF EXPANSION AND CELL PRODUCTION DYNAMICS UNDERLYING GROWTH RATE REGULATION IN ROOTS OF ARABIDOPSIS THALIANA.

Corine M. van der Weele, Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, cvdweele@wam.umd.edu

Plants live in a variable environment to which they regulate their growth and development. Plant growth is often limited by water deficiency, typically though roots are less affected than shoots. Shoots stop growing at minor stress while roots are capable of continuing growth, even under severe drought conditions. Although root growth rate is reduced, the continued growth does imply regulation of the processes involved. Growth is the production and expansion of cells. I studied the role of both processes in regulation of growth by exposing seedlings of Arabidopsis thaliana to constant levels of water deficit.
Seedlings were grown on an agar-solidified nutrient media containing high molecular weight polyethylene glycol to mimic drought conditions. A moderate stress treatment increased root growth rate for the first few days and under severe water deficit root growth was decreased compared to well-watered controls. The increased growth rate under moderate stress was paralleled by longer cells and higher cell production rate, while under severe stress cell length was the same as under well-watered conditions and cell production was reduced by half.

Cell production can be altered either by changes in the number of dividing cells or by changes in cell division rate.

SUPPRESSION OF RNA SILENCING IN PLANTS

Allison Mallory, Lewis Bowman, and Vicki Vance, Department of Biological Sciences, University of South Carolina, Columbia, SC 29212 USA

RNA silencing is a remarkable type of gene regulation based on sequence-specific targeting and degradation of RNA. The term refers to related pathways found in organisms as diverse as fungi (quelling), plants (post-transcriptional gene silencing, PTGS), protozoans, and a variety of animals including *C. elegans*, *Drosophila*, and mice (RNA interference, RNAi). In these organisms, the process is characterized by conserved genes and biochemical features. One key conserved feature is that the induction of RNA silencing involves dsRNA. In plants, RNA silencing may have evolved as a defense against viruses, many of which replicate via dsRNA intermediates. Consistent with this idea, a number of plant viruses encode suppressors of silencing. Here we report studies using one such suppressor of silencing, the helper component proteinase (HC-Pro) of potyviruses, as a tool to understand the mechanism of gene silencing. We show that HC-Pro suppresses silencing induced by three different classes of transgene, in each case eliminating the accumulation the short interfering RNAs (siRNAs) that mediate sequence-specific RNA degradation. In contrast, the accumulation of two other classes of small RNAs is enhanced in plants expressing HC-Pro. We have identified several cellular proteins that interact with HC-Pro in the yeast two-hybrid system. Studies of the role of these proteins in RNA silencing are providing clues about the mechanism and regulation of the silencing pathway. The emerging view is that RNA silencing is part of a sophisticated network of interconnected pathways for cellular defense, RNA-surveillance, and development, and may become a powerful tool to experimentally manipulate gene expression.

Maize BMS cultured cell lines survive with massive plastid gene loss

A. Bruce Cahoon, Katherine A. Cunningham, Thomas J. Bollenbach, and David B. Stern

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As part of developing an *ex planta* model system for the study of maize plastid and mitochondrial gene expression, we carried out preliminary experiments with an established Black Mexican Sweet (BMS) suspension cell line. Although our initial assumption was that its organelle biochemistry would be similar enough to normal *in planta* cells to facilitate future work, we found that the plastid genome carried large deletions, including genes previously reported to be indispensable for cell survival. By our estimates, as much as 70% of the wild-type maize plastid genome is missing in this line. Two additional BMS lines showed either a wild-type cpDNA pattern or an intermediate state of gene loss, suggesting that clonal lines are rapidly evolving. Gene expression profiles of BMS cells varied dramatically from wild-type whole plants, but resemble those of albino plants lacking plastid ribosomes. In spite of the lack of plastid gene expression BMS cells appear to import proteins from the cytoplasm in a normal manner. We conclude that the retained regions of the BMS plastid genomes along with other published accounts point to a single universally preserved set of tRNA genes and that deleted regions may offer telling examples of the requirement for certain plastid genes for plant cell survival. We also suggest that at least one of the BMS lines has an active plastid localized nuclear encoded RNA polymerase making this line(s) useful for continued analysis of plastid gene expression.

PROTEIN INFORMATION RESOURCE FOR FUNCTIONAL GENOMICS AND PROTEOMICS

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The human genome project has revolutionized the practice of biology and the future potential of medicine. With the accelerated accumulation of high-throughput genomic and proteomic data, computational approaches are increasingly important for deriving scientific knowledge and hypotheses. There is a pressing need to develop advanced bioinformatics infrastructure for biological knowledge discovery. As an integrated public resource of protein informatics, the Protein Information Resource (PIR) provides many databases and analytical tools to support genomic and proteomic research and scientific discovery. The Protein Sequence Database (PSD) is the major annotated protein database in the public domain, containing about 280,000 sequences covering the entire taxonomic range. To provide high quality annotation and promote database interoperability, the PIR uses rule-based and classification-driven procedures based on controlled vocabulary and accepted ontologies, and includes evidence attribution to distinguish experimentally determined from predicted protein features. PIR-NREF, a non-redundant database containing almost 1,000,000 proteins from PIR-PSD, Swiss-Prot, TrEMBL, GenPept, RefSeq, and PDB, provides a timely and comprehensive sequence collection with source attribution for protein identification, ontology development of protein names, and detection of annotation errors. The iProClass database addresses the database interoperability issues arising from the voluminous, heterogeneous, and distributed data. It provides comprehensive family relationships and functional and structural features for about 800,000 proteins in PIR-PSD, Swiss-Prot, and TrEMBL, with rich links to over 50 databases of protein families, functions, pathways, protein-protein interactions, post-translational modifications, structures, genomes, ontologies, literature, and taxonomy. An integrated protein knowledgebase, connecting the underlying data warehouse and sequence analysis and data mining tools with graphical user interfaces, is being developed for large-scale gene expression and proteomic data analysis, functional categorization, and pathway identification. The PIR databases are implemented in an object-relational database system and accessible from our web site (http://pir.georgetown.edu) for exploration of proteins and their comparative analysis. It helps users to answer complex biological questions that may typically involve querying multiple sources and detect interesting relationships among protein sequences and groups. Such knowledge is fundamental to the understanding of protein evolution, structure, and function, and crucial to functional genomic and proteomic research.

The PIR is supported by the NIH grant P41 LM05798, iProClass is supported by the NSF grants DBI-9974855 and DBI-0138188, and the Protein Name Ontology project is supported by the NSF grant ITR-0205470.

SEARCHING FOR STRUCTURE IN MULTIVARIATE DATA

F. James Rohlf, Dept. of Ecology and Evolution, SUNY, Stony Brook, NY 11794
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Multivariate techniques for searching for patterns in multivariate data will be discussed. The properties of various types of cluster analyses, ordination analyses, and methods based on graph theory will be described both in terms of their interpretations and in terms of their practicality for application to high-dimensional datasets such as found in studies of gene expression.

Characterisation of Ecuadorian cacao (***Theobroma cacao*** L.)

[1]Rey Loor, [2]James A. Saunders, [1]Freddy Amores

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ABSTRACT

Native Ecuadorian chocolate trees (Nacional variety) are in danger of being eradicated from Ecuador because of their susceptibility to widespread fungal diseases of cacao and farmer selection for others varieties. More than 95% of the area that contained Nacional cacao cultivated has been replaced with cacao from other countries. For Ecuador, it is a priority to identify and preserve the genotype of the native varieties due to the superior flavor properties for production of the quality chocolate (the famous "ARRIBA" flavor). Molecular techniques are very useful tools in the genetic identification and characterization of cultivars prior to the implementation of genetic breeding programs. The present work utilized simple sequence repeats (SSRs), using 15 different primers that have been proposed as international molecular standards for cacao to characterize Ecuadorian cacao genotypes. Fluorescent peaks from DNA fragment analysis were obtained using capillary electrophoresis (ABI 310 genetic analyzer) and were translated into binary data (i.e. presence (1), absence (0)) to perform an UPGMA (Unweighted Pair Group Method, Arithmetic average) cluster analysis using NTSYS software (Numerical Taxonomy System).

The dendrogram showing the genetic diversity among the cacao samples shows Ecuadorian Nacional genotypes grouped together and separated from the Amazonian varieties. No identical individuals were found among the group of accessions. Using SSR DNA fingerprinting methodologies we can get useful information for the development of breeding programs through the assessment of homozygosity, heterozygosity and individual relatedness.

FIRST ASSESSMENT OF GENE EXPRESSION IN THE SOYBEAN RUST *Phakopsora pachyrhizi*

M.L POSADA and R.D. Frederick. USDA-ARS Foreign Disease-Weed Science Research Unit, Fort Detrick, MD 21702.

Soybean rust is caused by the obligate fungal pathogen *Phakopsora pachyrhizi* Sydow. A unidirectional cDNA library was constructed in the plasmid pSPORT1 using mRNA isolated from *P. pachyrhizi* urediniospores germinating on a water surface. Single pass sequencing of 908 clones revealed that 404 sequences displayed significant similarities (E*value*$<10^{-05}$) to sequences deposited in public databases. The remaining 504 sequences showed no significant or no similarities to protein database entries. 488 unique ESTs were identified. Among genes with assigned function, approximately 20% were involved in primary metabolism, 7.5% in gene/protein expression, 5.5% in cell structure and growth, 4.5% in cell division, 3.5% in cell/organism defense and 3% in cell signaling/cell communication. Approximately 56.5% of the identities found were to hypothetical proteins and proteins with unknown function.

SA- AND NO-MEDIATED SIGNAL TRANSDUCTION IN PLANT DISEASE RESISTANCE

Daniel Klessig , Boyce Thompson Institute for Plant Research at Cornell University, Ithaca, NY

Studies during the past decade have rigorously established that salicylic acid (SA) plays a critical, multifaceted role in plant disease resistance. To help elucidate the mechanisms of SA action, we have identified several tobacco proteins which interact with SA. These include catalase and ascorbate peroxidase. SA inhibits these two major H2O2-scavenging enzymes. Another SA binding protein, the chloroplastic SABP3, is carbonic anhydrase. It also has antioxidant activity. SABP2 is a very low abundance protein with high affinity for SA (Kd=90nM). It has been purified >24,000 fold and the sequence of its encoding gene suggests it is a lipase.

We have used mutant analyses in *Arabidopsis* to identify several more potential components in the SA-mediated pathway. Among these mutants are those which exhibit constitutive expression of the SA- and pathogen-induced *PR* genes and enhanced resistance to pathogens (e.g. *cep* and cpr22). Another group are suppressor mutants which overcome salicylate insensitivity of our *sai1/npr1-5* mutant (e.g. *ssi1* and *ssi2*). Recently, we showed that *ssi2,* which activates the SA-mediated defense pathway but suppresses the jasmonic acid/ethylene-mediated defense pathway, alters the activity of a fatty acid (stearic acid) desaturase. Moreover, the product of this stearoyl desaturase, oleic acid or a derivative of it, appears to act as a signaling molecule which is required for activation of several jasmonic acid-mediated defenses.

Nitric oxide (NO), which plays a key role(s) in innate immune and inflammatory responses in animals, also participates in the tobacco resistance responses to TMV. Following infection, a NO synthase-like activity rises, leading to *PR-1* activation. Several critical players of NO signaling in animals are also operative in plants including guanylate cyclase, aconitase, and the second messengers cGMP, cADP ribose and Ca2+. Interestingly, SA and NO appear to share several common targets.

Related Publications: Klessig, D.F., Durner, J., Zhou, J.M., Kumar, D., Navarre, R., Zhang, S., Shah, J., Wendehenne, D., Trifa, Y., Noad, R., Kachroo, P., Pontier, D., Lam, E. and Silva, H. (2000) NO and salicylic acid signaling in plant defense. Proc. Natl. Acad. Sci. USA. 97: 8849-8855.

Navarre, D., Wendehenne, D., Durner, J., Noad, R. and Klessig, D.F. (2000) Nitric oxide modulates the activity of tobacco aconitase. Plant Physiol. 122: 573-582.

Kachroo, P., Shanklin, J., Shah, J., Whittle, E.J. and Klessig, D.F. (2001) A fatty acid desaturase modulates the activation of defense signaling pathways in plants. Proc. Natl. Acad. Sci. USA. 98:9448-9453.

Yoshioka, K., Kachroo, P., Tsui, F., Sharma, S.B., Shah, J. and Klessig, D.F. (2001) Environmentally-sensitive, SA-dependent defense response in the *cpr22* mutant of Arabidopsis. Plant J. 26:447-459.

PATTERNS OF TRANSLATION THAT LEAD TO RAPID DEVELOPMENT DURING SPERMIOGENESIS IN *Marsilea vestita*

Stephen M. Wolniak, Vincent P. Klink, Chiawei Tsai, Corine M. van der Weele, and Faten Deeb. *Department of Cell Biology and Molecular Genetics, University of Maryland, College Park*

Spermiogenesis in the water fern *Marsilea vestita* is a rapid process that is activated by placing dry microspores into water. Populations of male gametophytes develop synchronously, and each gametophyte develops within the microspore wall. The microspore contains a single cell that initiates a series of nine successive mitotic division cycles to produce 39 cells - one prothallial cell, six sterile jacket cells and 32 spermatids. The cell division planes are precise. Both position and distinct compositional differences between spermatogenous cells and sterile cells underlie cell fate determination in the gametophyte. As the division phase nears completion, a novel cytoplasmic particle, known as a blepharoplast, forms in each spermatocyte, serves as the centrosome for the spindle of the last division, and then serves as a site for the *de novo* formation of basal bodies. During the next 5.5 h, each spermatid assembles a complex cytoskeleton that facilitates extensive elongation and coiling of the cell body and the nucleus. The basal bodies serve as templates for the formation of ciliary axonemes. The process reaches completion in ~11 h with the release of 32 spermatozoids from each gametophyte. Each spermatozoid is a coiled cell that possesses ~140 cilia.

For the past several years, we have been interested in the processes of basal body formation and cell fate determination in the male gametophytes of *M. vestita*. We have found that rapid development of the male gametophyte is controlled at a post transcriptional level; the dry microspore contains large quantities of stored proteins and stored mRNAs, and the translation of stored transcripts controls the rate and extent of development. For the assembly of the cytoskeleton and the ciliary apparatus, the fern uses a set of highly conserved genes that have been identified in fungal and animal cells. We have found that specific mRNAs (*e.g.*, centrin, cyclin A, cyclin B, -tubulin, P28, kinesin) are translated at specific times during development, and translation of these transcripts is restricted in distribution to the spermatogenous cells. We have developed RNAi strategies to disrupt individual mRNAs in the gametophytes, and have shown that centrin is an essential component in the blepharoplast; in the absence of centrin, basal body formation will not occur. We have disrupted the cell division cycles in the gametophyte using pharmacological probes and with dsRNAs derived from cyclin A and cyclin B. Even in the absence of cell divisions, centrin is made at its normal time, but blepharoplasts fail to form. In the absence of blepharoplasts, basal body assembly is completely blocked. We have begun to look at controlling factors for the patterns of mRNA and protein distributions during cell fate determination. In a screen of our gametophyte cDNA library, we found a homolog for mago nashi, a protein involved with axis determination and gonad formation in animals. The protein encoded by our *Mv*-mago cDNA is more than 70% identical with mago nashi proteins from a variety of animals. The destruction of stored *Mv*-mago mRNA in the gametophyte by RNAi treatment results in a change in the plane of cell divisions in the gametophyte. In addition, the *Mv*-mago protein is apparently involved in the control of spatial distributions of certain mRNAs and the locations of translational activities in the gametophyte. Thus, this protein appears to play multiple roles in cell fate determination during spermiogenesis.

J-DOMAIN PROTEINS IN TOMATO AND STRAWBERRY THAT ARE HEAT SHOCK PROTEINS AND ARE EXPRESSED IN REPRODUCTIVE TISSUES

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Eucaryotes have a large number of proteins containing a conserved J-domain, corresponding to the N-terminal 75 amino acids of *E. coli* heat stress protein, DnaJ. DnaJ homologues in eucaryotes are also referred to as Hsp40. In plants, J-domain proteins have been implicated as molecular chaperones in responses to a wide range of environmental stimuli. They appear to have additional, unknown, functions however, and there are 89 J-domain containing proteins belonging to 51 distinct sub-families in Arabidopsis (Miernyk, 2001). A full-length cDNA (leDJA1) from tomato encoding a 46.8 kD protein with over 70% amino acid identity to known plant Hsp40 sequences was identified. Low levels of leDJA1 transcript were seen in seedlings, young leaves, and stems during normal plant growth. In comparison, leDjA1 mRNA is present in great abundance in fruit from early immature green stage and continuing throughout ripening, and increases upon heat treatment of young leaves. Antibodies specific to a highly conserved domain or the carboxyl (variable) region of leDjA1 were raised. Preliminary experiments with these antibodies showed that in strawberry, as in tomato, specific J-domain proteins are highly expressed in reproductive structures beginning early in floral bud development. We are working to identify genes encoding the reproductive structure J-domain proteins and the heat induced Hsp40s in tomato and strawberry in order to understand the mechanisms by which the plant cell uses the conserved J-domain motif for such apparently diverse functions.

CHARACTERIZATION OF AN ARABIDOPSIS GENE ENCODING A FIP1 HOMOLOG INVOLVED IN POLYADENYLATION

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The protein factors involved in the 3′-end formation of eukaryotic pre-mRNAs are highly conserved throughout evolution. Database BLAST searches of the Arabidopsis genome, yields homologs to several yeast and mammalian polyadenylation factors. Here we report the characterization of an Arabidopsis gene encoding a homolog to the yeast and human Fip1 subunit, named AtFip1. A GST-fusion protein of the first 483 amino acids of AtFip1, stimulates the non-specific activity of poly(A) polymerase *in vitro.* In a yeast two-hybrid assay, using the same amino-terminal clone (amino acids 1-483), AtFip1 is found to interact with the Arabidopsis cleavage and polyadenylation specificity factor subunit 30 (CPSF30) and cleavage stimulatory factor subunit 77 (CstF77) homologs.

Taken together, these results suggest that AtFip1 is a genuine subunit of the plant polyadenylation apparatus. Currently, clones of the carboxy-terminal region of AtFip1 are being cloned in GST and yeast two-hybrid expression vectors for further interactive analysis with poly(A) polymerase and other polyadenylation homologs. The characterization of AtFip1 will lead to better understanding of the biochemistry of poly(A) polymerase activity, and mRNA processing and metabolism in plants.

Plant metabolic engineering: From the Pharmacy to the Farm

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Vascular plants are literally rooted in their environment and adapt to change by shifting their growth, development, and biochemistry. Plant metabolic engineering seeks to exploit this broad plasticity to increase or decrease the content of specific metabolites in transgenic plants. Our research has focused on engineering pathways for high value pharmaceuticals including opiates (morphine, codeine, and thebaine) and anticancer indole alkaloids (vincristine, vinblastine and camptothecin). We have also shown that transfer of genes encoding early pathway enzymes into heterologous species imparts enhanced resistance to insects suggesting a broader application of this approach to agriculture. The ability of gene transfer technologies to bridge large phylogenetic distances not achievable by traditional breeding offers the opportunity to shuffle natural product pathways and confront plant diseases and pests with new chemistries to which they have not co-evolved.

Mucosal Immunology and the Mean Green Vaccine

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Vaccines are highly effective in preventing many diseases and have become a mainstay of modern medicines. However, traditional vaccines (generally killed or attenuated disease organisms) have limitation in meeting current global vaccine needs for common infectious diseases and the new challenges associated with defense against bioterrorism. Plants show promises as bioproduction (and potentially delivery) systems for vaccine antigens that would address key limitations in cost, scalability, and safety. However, many recombinant antigens are not highly effective immunogens compared to the large complex antigens presented by whole organisms. We have identified a plant-based immune adjuvant, MAC1 (Mucosal *A*djuvant/Carrier 1), which greatly facilitates mucosal delivery and immunogenicity of fused antigens. MAC1 is itself a non-toxic lectin that enhances both humoral and mucosal antibody responses to vaccine antigens. Our results have significant implications for development of plant-based vaccines for intranasal or oral administration.

Rapid mRNA decay mechanisms: Insight from genetic and genomic approaches

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Highly unstable mRNAs are of particular interest in eukaryotes because they allow organisms to respond rapidly to internal and external stimuli. One pathway for rapid mRNA decay in Arabidopsis is mediated by an mRNA instability sequence called DST that is highly conserved in the 3' untranslated regions of unstable Small-Auxin-Up-RNAs (*SAUR*s). A genetic selection for mutants with defects in the pathway led to the isolation of *dst1*, *dst2* and *dst3*. These mutants elevate the level of two transgene mRNAs, *HPH-DST* and *GUS-DST,* and an endogenous DST-containing mRNA called *SAUR-AC1*. To understand more about the molecular phenotypes of *dst1*, we compared gene expression in the mutant with that of the parental line using DNA microarrays of 11,000 Arabidopsis ESTs. These studies identified new genes with altered mRNA abundance in the mutants, a number of which contain DST-like elements and are presumably primary targets of the *dst1* defect. About a third of the transcripts that change in *dst1* are circadian regulated. This is higher than would be expected by chance and may indicate an association of the DST-mediated decay pathway and the circadian clock. Interestingly, we also observed a circadian association in another study aimed at identifying the most inherently unstable mRNAs represented on the 11K microarrays. By hybridizing the arrays with probes corresponding to RNA before and after transcription was inhibited for two hours, we found that at least 1% of the transcripts represented decayed with half-lives of less than 60 minutes. These unstable transcripts encode proteins that are predicted to participate in a broad range of cellular processes, with transcriptional functions being over-represented relative to the whole Arabidopsis genome annotation. Analysis of public microarray expression data for these genes argues that mRNA instability is of high significance during plant responses to mechanical stimulation and is associated with specific genes controlled by the circadian clock. Supported by NSF, USDA and DOE.

EXTRACELLULAR RIBONUCLEASE ACTIVITY NEGATIVELY AFFECTS GROWTH OF ARABIDOPSIS PLANTS

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Secreted ribonucleases of the RNase T_2 family have long been considered to be recycling enzymes that degrade nucleic acids in dead cells or the surrounding medium for utilization by surviving cells. This concept was based on their predominantly extracellular or vacuolar localization and their expression patterns, including in response to phosphate starvation (1, 2). However, the cytotoxicity of S-RNases, the presence of RNase T_2 enzymes in virus, and their absolute conservation in all kingdoms and almost all organisms so far examined (1, 2) suggest that these enzymes may have a more essential role that has been conserved in all organisms throughout evolution. In a previous work (3), we showed that yeast cells lacking Rny1, the only T_2 RNase family member present in *S. cereviseae*, are larger than WT cells and have temperature and osmosensitive phenotypes. Here we show that Arabidopsis plants with a mutation in *RNS1*, one of five RNase T_2 family genes in this plant, have an altered growth phenotype, which is most evident in roots. Mutant plants have roots that are 30% longer than WT plants. Plants overexpressing this extracellular enzyme have roots that are shorter than WT. Our results indicate that extracellular RNase activity negatively correlates with plant growth. Cytological analysis showed that the cellular organization of the root meristem is normal. However, analysis of cell elongation by *in vivo* imaging indicated that the difference in root length is probably due to an increased cell elongation rate in the mutants. Staining of live roots with the pH-sensitive dye Oregon Green suggests that mutant cells also are more permeable and/or have increased intracellular pH. Hypotheses on the mechanism of action of secreted ribonucleases in controlling cell growth will be discussed.

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SEQUENCE DIVERSITY AND LONG RANGE LINKAGE DISEQUILIBRIUM AROUND THE MAIZE Y1 LOCUS

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The extent of linkage disequilibrium (LD) in the maize population has important consequences on the usefulness of association mapping approaches for correlating genotypes with phenotypic variability in maize. Recent reports in maize have described a rapid decline in LD, ranging from 100-200 bp to approximately 1500-2000bp [Tenaillon *et al.*, 2001; Remington *et al.*, 2001; Thornsberry *et al.*, 2001]. However, human studies have shown that the level of LD is variable across the genome, as a result of selection, chromosomal location, etc. To further evaluate the patterns of LD and sequence diversity in maize, the sequences within and surrounding the *Y1* locus were analyzed. This gene, which is positioned on chromosome six, determines the endosperm color of maize kernels and is thought to have undergone repeated selection for the yellow endosperm phenotype due to its higher nutritional content. Taking advantage of the published sequence of *Y1* and a 1 Mb BAC contig with this locus at a central position [Morgante, 2001], primers were chosen to amplify various portions of the *Y1* gene and its surrounding genomic regions. The PCR products obtained from a set of 78 inbreds were directly sequenced, and all polymorphic sites within the amplicons were identified. Significantly higher nucleotide diversity was found in the white endosperm inbreds as compared to the yellow endosperm inbreds at this locus, and considerable linkage disequilibrium was apparent within the 6 kb region of *Y1,* as evidenced by a common haplotype shared by the yellow endosperm inbred lines. In addition, the low copy regions that were identified in the large *Y1*-containing BAC contig evinced similar patterns of diversity up to 300kb away from the *Y1* locus. Therefore, the level of LD in this study extends much further than was observed previously, perhaps because this gene has been under heavy selection pressure.

Transcriptional regulation, tissue-specific and developmental expression of 1-SST (sucrose: sucrose 1-fructosyl transferase) from *Taraxacum officinale*

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1-SST is the key enzyme initiating fructan synthesis in Asteraceae. Using RT-PCR with 1-SST specific primers, we isolated the 1-SST cDNA from dandelion (*Taraxacum officinale).* At the amino acid level, the cDNA showed very high homology to other Asteracean 1-SST 's (*Cichorium intybus* 86%, *Cynara scolymus* 82%, *Helianthus tuberosus* 80%) but homology to 1-SST from *Allium cepa* (51%) and *Aspergillus foetidus* (31%) was much lower.
We analysed fructan concentrations, 1-SST enzymatic activities, 1-SST mRNA concentrations (Northern blots) and 1-SST protein concentrations (Western blots) in different tissues or organs from flowering *Taraxacum* plants (second year of growth): stalk, receptacle, intervenal leaf parenchyma, leaf veins, root phloem and root xylem. A good correlation could be found between Northern and Western blots pointing out that 1-SST is regulated at the transcriptional level. At the pre-flowering stage, 1-SST mRNA concentration was higher in the root phloem compared to the xylem resulting in higher 1-SST activities and higher fructan concentrations in the phloem. Fructan localization studies indicated that fructan is preferentially stored in clusters of phloem parenchyma cells in the immediate surroundings of the secondary phloem. However, inulin crystals also appeared to be present within xylem vessels.

GROWTH AND OXALIC ACID PRODUCTION IN LIQUID CULTURE BY ISOLATES OF *SCLEROTINIA MINOR:*

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A previous study of *Sclerotinia minor* reported correlation between aggressiveness on susceptible peanut and colony size. A color change on pH indicator plates two to three days after transfer was also observed (Hollowell *et al.*, 2001). The authors reported that lesion size on leaves was correlated with mycelial growth in broth culture, but not oxalic acid production after two or three days in culture. We have undertaken further studies to examine growth characteristics and oxalic acid production of the fungal isolates during two weeks in liquid culture. In addition, we have compared different detection methods for quantifying oxalic acid levels in culture medium. Our comparison of different isolates of *Sclerotinia minor* confirmed that mycelial growth in potato dextrose broth is correlated with aggressiveness. However, levels of oxalic acid in culture medium over the same time period varied considerably and could not be used as reliable predictors of aggressiveness. Culture medium from the moderately aggressive isolate NC22 contained 3 times the amount of oxalic acid compared to NC13 based on mycelial dry weight after ten days (4.08 mg/g vs.1.38 mg/g, respectively). To investigate whether oxalic acid detection methods influenced the accuracy and reproducibility of these findings, we compared two different protocols for measuring oxalic acid in culture medium. We tested high performance liquid chromatography (HPLC) and a commercially available kit, designed for detection of urinary oxalate, for characteristics such as sensitivity, accuracy, cost, and ease of use. HPLC (Shodex RSpak KC-811 column) accurately quantifies oxalic acid over a broader range of concentrations than the spectrophotometric assay in the kit (0.1 - 200 mg for HPLC vs. 0.1 - 20 mg for the kit). Although reagents for the spectrophotometric assay are more expensive, HPLC is more labor intensive, requires the availability of the appropriate instrumentation, and involves extraction with organic solvents.

MOLECULAR ANALYSIS OF *HSP90*, A MULTI-FACETED GENE INVOLVED IN THE GROWTH AND DEVELOPMENT OF FREE-LIVING AND PLANT PARASITIC NEMATODES.

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Plant parasitic nematodes cause in excess of $100 billion of global crop losses each year. Cyst nematodes (*Heterodera spp.*) are endoparasitic root-feeding nematodes, and the soybean cyst nematode (SCN), *Heterodera glycines*, causes substantial losses in soybean yield in the U.S.A. as well as throughout the world. The most successful method for controlling SCN infestation involves the use of resistant cultivars, but because soybean populations are constantly changing, the durability of this strategy may be threatened. Crop rotation may be economically unfavorable to growers, and several nematicides have been or soon will be banned from use. Therefore, an urgent need exists for the development of novel, biologically based control strategies. The developmentally arrested juvenile (J2) stage of SCN comprises a vulnerable point in the nematode life cycle, and the genes that control nematode development in response to environmental changes provide attractive targets for disruption. One such target is the *hsp90* gene. In the free-living nematode *Caenorhabditis elegans*, the *hsp90* gene known as *daf-21* is involved in the dauer pathway, an alternative developmental pathway that occurs as a result of extreme environmental conditions such as starvation and overcrowding. HSP90 molecular chaperones regulate the correct folding, activation and assembly of specific target proteins that control normal cellular development and metabolism. The objective of this study is to investigate the role of HSP90 in the growth and development of SCN and to determine the effect of HSP90 disruption on nematode development. We are using the yeast two-hybrid system to characterize the interactions between *H. glycines* HSP90 and other known members of the *C. elegans* dauer pathway. In addition we are studying interactions between HSP90 and HCH-1, a putative HSP90 co-chaperone that is involved in nematode hatching. Finally, we are also investigating the effects of geldanamycin, a naturally occurring compound that binds to and inhibits HSP90, on the growth and development of *C. elegans* as a model for later studies on *H. glycines*.

BIOLISTIC TRANSFORMATION OF SUGAR BEET CELL SUSPENSIONS

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Nonproprietary sugar beet transformation methods are plagued by low transformation frequencies and lack of reproducibility. In efforts to optimize the methods, we established highly embryogenic sugar beet cell suspension cultures for transformation by the particle bombardment method. Callus obtained from leaf discs of greenhouse-grown FC607 plants was propagated in liquid medium for 2 weeks, sieved, and then plated for 1 day on agar medium prior to bombardment. After 2 to 5 days callus was passed to selection medium containing either kanamycin or paromomycin or no antibiotics. Transformation vectors carried the reporter gene *uidA* (GUS) fused to either the osmotin (Osm) or proteinase inhibitor II (Pin2) gene promoter, or the EGFP gene under control of the double 35S promoter. Transient GUS expression monitored 2 days after bombardment showed 900 to 3000 blue units per bombarded plate of 0.2 g of suspension cells. Transient EGFP expression visualized with epifluorescence microscope showed similar number of fluorescent cells per bombarded plate. Both GUS and EGFP expression decreased significantly during the initial 14 days of culture. Stably transformed GUS (+) calli were obtained as early as 3 weeks following bombardment at a frequency of 0.25 - 9 calli per bombarded plate but no GUS (+) shoots regenerated even though plated control cell suspensions were highly embryogenic. Further modulation of the plant growth regulator composition in the media may promote the regeneration of transgenic shoots.

RECENT UPDATES TO THE SOYBEAN GENOMICS AND MICROARRAY DATABASE (SGMD).

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Abstract:

The soybean genomics and microarray database (SGMD) was established in 1999 to serve as a sequence and microarray database for the Soybean Genomics and Improvement Laboratory (SGIL), Beltsville Agricultural Research Center (BARC) and collaborators. It serves both as a sequence repository, holding DNA sequences for numerous EST's, and also as a microarray experiment database. EST sequences stored in SGMD include cDNA's derived from soybean roots infected to soybean cyst nematodes and cDNA's derived from soybean cyst nematodes obtained through the NSF-funded nematode EST project. SGMD allows scientists to explore the expression levels of the EST clones in roots of susceptible and resistant soybean cultivars infected with the soybean cyst nematode, and to correlate expression levels with function. SGMD is a relational database built on SQLServer2000 and incorporates the minimal information about a microarray experiment (MIAME) guidelines set forth by the microarray gene expression database (MGED) group. Recent updates to the database are discussed, which include updates to the database structure, the data that it contains and the new web sites and user interfaces that have been developed recently. Among the many updates to SGMD is the inclusion of a Soybean Cyst Nematode (SCN) database and the addition of time series microarray experiments and web based interfaces to query the data across the time points and/or between experiments. SGMD can be accessed from http://bldg6.arsusda.gov/benlab.

USE OF MOLECULAR GENETIC AND GENOMIC APPROACHES FOR THE STUDY OF COLD HARDINESS IN BLUEBERRY

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Environmental stresses, including low temperature extremes, reduce crop yields and impact the profitability and competitiveness of U.S. producers. The U.S. is the world's leading blueberry producer. The blueberry industry in the U.S. suffers from a lack of winter hardy and spring-frost resistant cultivars. In order to increase our understanding of the genetic control of cold hardiness and ultimately use this information to develop more cold hardy cultivars, our laboratory has been cloning and characterizing expression of cold-responsive genes from blueberry. We have used a molecular genetic approach to identify genes that are up-regulated during cold acclimation and, more recently, have begun using a genomic approach to identify and characterize a much larger group of genes expressed during cold acclimation. Previously, we identified a group of dehydrins (proteins induced by environmental stimuli that have a dehydrative component such as freezing and drought stress) of 65, 60, and 14 kDa that are the predominant up-regulated proteins in cold acclimated floral buds of blueberry. Recent expression studies indicate that (1) blueberry dehydrins are induced by cold stress in all organs but by drought stress in mainly stems; (2) dehydrin accumulation correlates positively with cold tolerance but not with drought tolerance; and (3) dehydrin expression in blueberry cell suspension cultures is different from that in whole plants. Several cDNA clones representing members of the dehydrin gene family have been isolated including full-length clones for the 60 and 14 kDa dehydrins. Finally, a genomic approach to the study of cold-responsive genes in blueberry has been undertaken. EST (expressed sequence tag) analysis of a cDNA library representing genes that are expressed mid-winter, when plants have reached their maximum level of cold hardiness, is being used to develop EST-PCR (expressed sequence tag-polymerase chain reaction) markers for mapping purposes and to categorize the types of genes expressed during cold acclimation.

Differential Expression and Roles of the Vacuolar H^+-ATPase subunit c1 and c3 in Plant Growth

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The vacuolar H^+-ATPase (VHA) acidifies intracellular compartments and generates a proton electrochemical gradient to transport ion and metabolite across vacuolar and other membranes; although the potentially diverse functions of this complex pump in plant growth, development and adaptation are not understood. The multiple subunits that form a peripheral V_1 complex and an integral V_o complex are encoded by a single gene or by multiple genes in Arabidopsis. Of these, the 16kda subunit c of the V_o sector is encoded by the largest gene family with 5 members, Vha-c1 to Vha-c5. Analysis of promoter-Gus reporter gene showed c1 and c3 are differentially expressed. c1 is highly expressed in most tissues of the root and leaf, whereas c3 is expressed only in the root tip. In the flower, c1 is expressed in all flower parts including sepal, stamen, anther and stigma but not in the petal. In contrast, c3 is expressed only in pollen. In dark-grown etiolated seedlings, c1 is highly expressed in hypocotyls, but not in the cotyledons. In light-grown (blue or far red) seedlings, expression of c1 is high in cotyledons but not in hypocotyl. Thus c1 expression accompanies cell expansion, and its promoter activity is tissue-specific and developmentally regulated. To test in vivo function of each subunit c, homozygous plant containing ds-RNA constructs of c1 or c3 were analyzed. RT-PCR confirmed that 6 out of 10 independent transformants showed reduced levels of native RNA. Relative to wild type, roots of 7 d etiolated seedlings with dsRNA-c1 were shorter by 40%; surprisingly, the hypocotyls were only 15% shorter. Roots of plants with ds-RNA c3 were also inhibited though by only 20%. These results demonstrate that c1 and c3 subunits are differentially expressed, and that the V_1V_o-ATPase complexes formed by c1 or by c3 play differential roles in plant growth.

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SEASONAL DIFFERENCES IN TRANSCRIPT INITIATION FROM PROMOTERS OF TWO PEACH DEHYDRIN GENES:

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A peach genomic clone, G10a, carries two dehydrin genes in tandem. The first gene, *Ppdhn1*, represents a Y2K9-type dehydrin based on the presence of conserved 'Y' and 'K' domains. The second gene, *Ppdhn2*, encodes a Y2SK3-type. G10a encodes approximately 1100 bp 5' of the *Ppdhn1* translation start site and contains the full length promoter. Likewise, the region between *Ppdhn1* and *Ppdhn2* (1120 bp) contains the entire promoter for *Ppdhn2*. cDNAs were synthesized from total RNA isolated from peach bark tissue at various times during the year. These cDNAs served as templates for different primer combinations designed to detect transcripts initiating from various regions of each promoter. *Ppdhn1* transcripts from July, January and February bark appeared to originate predominantly from two regions of the *Ppdhn1* promoter, whereas transcripts from December, May and June bark apparently initiated from only one region. Although *Ppdhn2* transcripts were also detected from two regions of the *Ppdhn2* promoter in July, January and February bark, no transcripts were observed from bark isolated in August, December, March, May or June. These results suggest that multiple signals are acting on the two promoters at different times of the year and that some of these signals affect transcript abundance of the two *Ppdhn* promoters differently.

CHARACTERIZATION OF THE DST-MEDIATED DECAY PATHWAY IN *ARABIDOPSIS THALIANA* UTILIZING GENETIC TECHNIQUES IN CONJUNCTION WITH MICROARRAY TECHNOLOGY

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We have used DNA microarrays to expand our understanding of the *dst1* mutant of Arabidopsis. The *dst* mutants were originally isolated as specifically elevating the steady-state level and increasing the half-life of DST-containing transcripts. As such, they offer a unique opportunity to study rapid sequence-specific mRNA decay pathways in eukaryotes. These mutants show a 3- to 4-fold increase in mRNA abundance for two transgenes and an endogenous gene, all containing DST elements, when analyzed by RNA gel blot; however they show no visible aberrant phenotype. Using DNA microarrays, we were able to identify new genes with altered mRNA abundance in *dst1* in addition to verifying the increase in the transgene mRNA levels, used to isolate these mutants. RNA gel blot analysis confirmed the microarray data for all genes tested and was also used to catalog the first molecular differences in gene expression between the *dst1* and *dst2* mutants. These differences revealed previously unknown molecular phenotypes for the *dst* mutants that will be helpful in future analyses. Clustering analysis of genes altered in *dst1* exposed new co-expression patterns that prompt new hypotheses about the nature of the *dst1* mutation and a possible role of the DST-mediated mRNA decay pathway in plants. Additional microarray experiments with *dst2* are beginning to provide further insight into the potential role of the DST-mediated mRNA decay pathway in plants.

OLIGOMERISATION OF β-GLUCOSIDASES IN MAIZE AND SORGHUM

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β-glucosidases are a ubiquitous group of glycosyl hydrolases that catalyze the hydrolysis of β-glucosidic linkages of disaccharides, oligosaccharides and conjugated glucosides. They are known to play an important role in various biological processes like plant defense, phyto hormone activation, biomass conversion etc. Structural analysis of β-glucosidases belonging to the family 1 of glycosyl hydrolases reveals that the catalytically functional units of these enzymes have a quaternary structure. Previous studies with the dimeric maize β-glucosidase ZMGlu1 have revealed that the treatment of this enzyme with denaturing agents produces catalytically inactive monomers. Whether this loss of activity can be attributed to the disruption of the dimer structure or denaturation of the monomers is not known. We addressed this question in maize β-glucosidases by changing amino acids involved in oligomerisation (i.e. those at the dimer interface) by site directed mutagenesis. Mutation of one of the arginine residues involved in the formation of the salt bridges at the dimer interface has resulted in mutants with reduced activity as compared to the wild type. The wild type and mutant enzymes were analyzed by activity staining with fluorogenic and chromogenic substrates on native and SDS gels without denaturing them. The mutants show activity but with different migration rates as compared to the wild type on a native gel and fail to show any activity on a SDS gel, whereas the wild type shows activity on both gels. The differential migration rate can be attributed to the change in the charge of the mutant enzyme upon removal of a positively charged residue at the dimer interface. Activity assays of the mutants in the presence of different concentrations of SDS has revealed increased sensitivity to SDS and a decrease in activity with increasing concentration of SDS. The sensitivity of the mutants to SDS may be responsible for their lack of activity on SDS gels. Similar mutagenesis studies will be done on the two sorghum isozymes of β-glucosidase, dhurrinase-1 (Dhr1) and dhurrinase-2 (Dhr2). These studies would help us understand the importance of oligomerisation in β-glucosidases for their activity or for stability or both.

GENETIC ANALYSIS AND SSR TAGGING OF THE GENE CONDITIONING POWDERY MILDEW RESISTANCE IN THE TOMATO HYBRID DRW4409

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Powdery mildew has become a serious disease for both greenhouse and field tomatoes in Northern America and most tomato cultivars are susceptible to this disease. To perform marker-assisted selection to improve traits of interest such as disease resistance, microsatellite markers (SSR) are preferred DNA markers because of their properties of codominance, high level of polymorphism and the feasibility of automation. In this study, we used an F_2 population derived from the tomato hybrid DRW4409 to develop microsatellite markers for powdery mildew resistance. The F_2-derived F_3 lines were used for disease evaluation and genotyping the F_2 plants in terms of disease resistance for identifying SSR markers. Genetic analysis showed that the powdery mildew resistance appeared to be controlled by a major incomplete dominant gene. To develop SSR markers for this gene, we screened 158 SSR loci and found 12 SSR loci were polymorphic between the two parents and among the F_2 individual plants. All the SSR markers segregated in Mendelian fashion for a 1:2:1 ratio except for the marker LEta019, which showed a distorted segregation. Of these 12 SSR markers, one marker, LEat014, with simple sequence repeats of $(at)_9$, was linked to powdery mildew resistance at a map distance of 8.0 cM. Two additional linkage groups were also constructed with 5 SSR markers. The determination of the relationship of this powdery mildew resistance gene with the known gene *Ol-1* was attempted but we were unable to find polymorphisms between the two parents for the reported molecular markers linked with *Ol-1*.

THE ROLE OF HOST-PLANT ASSOCIATION ON THE GENETIC DIFFERENTIATION OF SYMPATRIC POPULATIONS OF *Aleiodes nolophanae* (Ashmed) (Hymenoptera: Braconidae)

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Research on hymenopteran parasitoids suggest that parasitoids might be specialized in searching for their hosts in particular plant species. If that specialization occurs, genotypic differences among parasitoids specialized in searching for hosts in different crops are expected. The hymenopteran parasitoid *Aleiodes nolophanae*, a parasitoid of green cloverworm (*Plathypena scabra*) populations occurring in soybean and alfalfa, was used to test if host plant differences influence parasitoid genetic differentiation. Wasps were obtained from green cloverworms collected from alfalfa and soybean fields in Maryland. AFLPs were obtained from wasp DNA and analyzed using capillary electrophoresis (ABIPRISM 300 hardware and software) and UPGMA cluster analysis (NTSYS software). We found that wasps collected from green cloverworms feeding on soybean were grouped together in a defined cluster, suggesting the existence of a soybean *A. nolophanae* genotype. The areas from which *A. nolophanae* specimens were collected in this study, presented more cultivated area of soybean than alfalfa. This difference in abundance might be responsible for the soybean genotype found. Current research is analyzing genotypes from wasps collected from places on which the soybean and alfalfa cultivated areas are the same and from places on which there is more alfalfa than soybean cultivated area, to see if the relative abundance of the host plant has an impact in *A. nolophanae* genetic differentiation.

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