

*Midwest ASPB Meeting
March 24/25, 2007
Biomedical Physical Sciences Building
Michigan State University
East Lansing, MI*



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Saturday, March 24, 2007

8:00 am Registration in the Atrium of the BPS building; poster setup
Coffee, Tea, Bagels and Cream Cheese

8:30 am Welcome and Information about East Lansing
Susanne Hoffmann-Benning

8:35 am Welcome and Introduction
Richard Sayre, Chair MWASPB 2007

8:50 am Welcome by Mike Thomashow
Director, DOE-Plant Research Laboratory
ASPB Immediate-past President

9:00 am – 10:30am **Session I talks (12 minutes plus 3 minutes questions)**
(Moderator Laura Olsen; University of Michigan)

9:00 am Xiaomin Yu, Yuqin Jin, Basil Nicolau, and Eve Wurtele
Expression of branched fatty acid genes in Arabidopsis

9:15 am Aaron Wyman, Preekamol Klanrit, John, P.M. Manalo, Ahson Ali,
Sheryl A. Walker, Carrie M. Anderson, Mary Alice Webb
*Isolation and characterization of proteins associating with
calcium oxalate crystals*

9:30 am Andriy Tovkach and Tzvi Tzfira
*Design, assembly and cleavage characteristics of zinc finger nucleases for
gene targeting in plants*

9:45 am Chuangsheng Mei, Callista Ransom, Robab Sabzikar, Yanfen
Zhai, and Mariam Sticklen
*Transformation of cellobiohydrolase gene from Trichoderma reesei into
tobacco and maize plants*

10:00 am Brandon Wojcik and Laura Olsen
*Investigation of the expression and localization of sHsp15 in
Arabidopsis thaliana*

10:15-10:30 am *Coffee break*

10:30-12:00 pm **Session II talks (12 minutes plus 3 minutes questions)**
(Moderator Richard Sayre, MWASPB chair)

10:30 am Hangsik Moon, Satish Rajamani, Sareena Singh, Ditmuth
Siritunga, and Richard Sayre

Four cysteines and a lysine residue in the ATP binding domain are important in the function of Fea1, the iron transporter from Chlamydomonas reinhardtii

- 10:45 am Uzoma Ihemere, Wai-Ting Chiu, and Richard Sayre
Increasing the bio-available iron in cassava root by incorporating Chlamydomonas FEA1 gene
- 11:00 am Ying Deng, Futong Yu, Mei Chen, Jonathan Frantz, Scott Heckathorn, and John Gray
Biomonitoring of boron micronutrient stress in Arabidopsis thaliana and Pelargonium X Hortorum
- 11:15 am Antony Chettoor, Kejian Li, Xueyuan Cao, and Philip W. Beecraft
The COP9 signalosome is involved in ACR4 receptor turnover
- 11:30 am Manli Davis, Kengo Morohashi, Rebecca Lamb, and Erich Grotewold
Plant infantry: Arabidopsis thaliana trichomes as defense organs
- 11:45 am Kanchan A. Pavangadkar, Michael F. Thomashow, and Steven J. Triezenberg
Function of coactivator proteins ADA2 and GCN5 in cold acclimation in Arabidopsis
- 12:00-1:00 pm** *Lunch (Woody's Oasis/ Mediterranean food)*
- 1:00-2:45 pm** **Session III talks** (12 minutes plus 3 minutes questions)
(Moderator Mike Thomashow; immediate-past president ASPB)
- 1:00 pm Lalita Patel and Marianne Laporte
Analysis of the KAT-1 promotor and NADP-malic enzyme in Arabidopsis thaliana
- 1:15 pm Paolo Sabatini, Guo-Qing Song, Ken Sink, James Flores, and Wayne Loescher
Anti-sensing polyol (mannitol) biosynthesis in celery decreases salt tolerance
- 1:30 pm Jackson Moeller, Jaime Dittman, Rico Caldo, Roger Wise, and Steve Witham
Comparative functional analysis of plant pathogen responsive genes in model dicot and monocot pathosystems
- 1:45 pm Weiqing Zeng, Yonghua Li, and Sheng Yang He
Identification and characterization of the Arabidopsis mutant scd2 (susceptible to coronatine-deficient Pseudomonas syringae pv. Tomato strain DC3118)

- 2:00 pm Eliana Gonzales-Vigil, Hui Chen, Gregg A. Howe
Identification of plant defense proteins that impair insect digestive physiology: threonine deaminase as a case study
- 2:15 pm Crystal E. Montgomery, Prem Kumar, and John Z. Kiss
The role of phytochrome C in gravitropism and phototropism
- 2:30 pm Diana Roberts and Sarah Wyatt
Potential role of auxin response factor 9 in gravity signal transduction
- 2:45-3:00 pm** *Afternoon Coffee break (coffee, tea, water, biscotti)*
- 3:00-4:00 pm** **Plenary speaker Dan Voytas**; Iowa State University
Plant Genome modification through homologous recombination
- 4:00-4:30 pm** **break / business meeting**
- 4:30-5:30 pm** **Session IV talks (12 minutes plus 3 minutes questions)**
(Moderator Sarah Wyatt; Ohio University)
- 4:30 pm David Cavalier, O. Lerouxel, O. Zabolina., L. Neumetzler, W. Abasolo, I. Burgert, M. Pauly, N. Raikhel, C. Wilkerson, and Ken Keegstra
Arabidopsis XT1 and XT2 encode xylosyltransferases involved in xyloglucan biosynthesis
- 4:45 pm Yizu Zhang, Jie Yang, and Allan M. Showalter
Functional characterization of lysine-rich AGPs by reverse genetics
- 5:00 pm Christopher J. Havran and Harvey Ballard
Evolutionary mechanisms of prezygotic isolation in two replicate sublineages of Hawaiian violets
- 5:15 pm Sarah M. Owens, Corrine A. Frankenfield, and Richard C. Moore
The evolution of young gene duplicates in Arabidopsis thaliana
- 6:00pm** *Dinner catered by Sindhu's Indian Cuisine*
- 7:00-9:00 pm** **Poster session (snacks and drinks)**

Odd-numbered posters 7:00-8:00 pm
Even-numbered posters: 8:00-9:00 pm

Sunday, March 25, 2007

- 8:15 am *Coffee, tea, doughnuts in the BPS Atrium*
- 8:45-9:00 am Greetings by Crispin Taylor

- 9:00-10:30 am** **Session V talks (12 minutes plus 3 minutes questions)**
(Moderator Crispin Taylor, Executive Director ASPB)
- 9:00 am Sachin Teotia and Rebecca S. Lamb
Two putative poly (ADP-ribose) polymerases, RCD1 and SRO1 play important, partially redundant roles in Arabidopsis
- 9:15 am Tiffany J. Dickerson and Susanne Hoffmann-Benning
Characterization of proteins from corn coleoptile epidermis that are involved in auxin-induced growth
- 9:30 am Yunjing Wang, Harvey E. Ballard, R. Ryan McNally, and Sarah E. Wyatt
Identification and characterization of floral genes in a cleistogamous species Viola pubescens (Violaceae)
- 9:45 am R.Ryan McNally, Yunjing Wang, and Sarah E. Wyatt
Identification of Sepallata floral gene orthologs in Viola Pubescens
- 10:00 am Nicola Harrison-Lowe and Laura Olsen
Autophagy protein 6 (ATG6) is required for post-microsporogenesis pollen development in Arabidopsis thaliana
- 10:15 am Anthony Schillmiller, Feng, Shi, Curtis Wilkerson, Dan Jones, and Robert Last
Functional genomic analysis of tomato (Solanum) trichomes
- 10:30-10:45 am** *Coffee break*
- 10:45-12:15 pm** **Session VI talks (12 minutes plus 3 minutes questions)**
(Moderator Wayne Loescher, MWASPB Campus Rep.)
- 10:45 am Tawanda Zidenga, Dimuth Siritunga, Paul Chavariaga, and Richard Sayre
Cyanide metabolism, protein production, and post harvest physiological deterioration in cassava
- 11:00 am E. Leyva-Guerrero and R.T. Sayre
Cytoplasmic and vacuolar expression of linamarase in cassava roots for protein content enhancement
- 11:15 am Yang Xu and Steven Rodermel
A plastid polyribonucleotide phosphorylase suppresses variegation in the Arabidopsis var2 mutant

- 11:30 am Andrea Braeutigam, Susanne Hoffmann-Benning, and Andreas Weber
Identification and characterization of Mep1, a novel plastid envelope protein
- 11:45 am Binbin Lu, Changcheng Xu, Koichiro Awai, and Christoph Benning
TGD3, an ATPase protein of Arabidopsis, functions in ER-to-plastid lipid trafficking
- 12:00-1:00 pm** *Lunch (sandwiches)*
- 1:00-2:00 pm** **Faculty talks** (12 minutes plus 3 minutes questions)
(Moderator Jianping Hu; Michigan State University)
- 1:00 pm Winfried S. Peters, William F. Pickard, Amy Q. Shen, and Michael Knoblauch
Forisomes- a unique ATP-independent contractile apparatus in the sieve tubes of the legumes
- 1:15 pm Tzvi Tzfira
Integration of Agrobacterium's T-DNA molecules into genetic double strand breaks
- 1:30 pm Debbie Swarthout, Emily Harper, Stephanie Judd, David Gonthier Timothy Stowe, and Thomas Bultman
Optimization of water-use efficiency by an endophytic fungus in Lolium arundinaceum
- 1:45pm** **Meeting will end with the presentation of student awards**

Plenary Talk

Plant genome modification through homologous recombination

Dan Voytas; Dept. of Genetics, Development & Cell Biology; Iowa State University

Engineered zinc-finger nucleases can stimulate high frequency gene targeting (homologous recombination) at specific genomic loci in plants (*Plant Journal*, 44:693). ZFNs consist of a Cys₂His₂ zinc finger domain engineered to bind a particular gene sequence and the non-specific nuclease domain of the FokI restriction enzyme. These artificial proteins introduce double-stranded breaks at specific DNA sequences and thereby greatly increase the rate of homologous recombination at the cleaved locus. ZFN-mediated gene targeting provides many uses in both basic and applied plant biology; however, the general application of this technology depends critically on the ability to design zinc finger domains targeted to any desired DNA sequence. To address this need, the Zinc Finger Consortium was established to promote continued research and development of engineered zinc finger technology. In initial work, the Consortium developed a unified, robust, and user-friendly zinc finger engineering platform (*Nature Protocols*, 1:1637). A comprehensive archive of plasmids was created that encode more than 140 well-characterized zinc-finger modules together with complementary web-based software for identifying potential zinc-finger target sites in a gene of interest. The Consortium also developed protocols for rapidly testing the DNA-binding activities of assembled multi-finger arrays in bacterial and yeast cell-based reporter assays as well as vectors for the expression of zinc finger nucleases in plants. Results will be presented on the use of ZFNs generated with Consortium reagents to modify native plant genes.

Oral Presentations

1. Expression of branched chain fatty acid genes in *Arabidopsis*

Xiaomin Yu, Yuqin Jin, Basil Nikolau, Eve Wurtele

Dept. of Genetics, Development and Cell Biology, Iowa State University, IA50011

Vegetable oils are composed of linear fatty acids. Although these oils provide desirable lubricant properties, the suboptimal oxidative stability at high temperatures and high pour point temperature of vegetable oils limit their applications. Esters of branched chain fatty acids can substantially improve these qualities. Either of two *Bacillus* genes (yhfB and yjaX), homologs of the *E.coli* and *Arabidopsis* KASIII enzymes, can confer branched chain fatty acid biosynthesis in *E.coli*, which does not normally produce these fatty acids. Our hypothesis is that introduction of yhfB and/or yjaX might alter fatty acid composition by inducing branched chain fatty acid biosynthesis in *Arabidopsis*. Both non-targeted and plastid-targeted expression of these two genes was conducted. Our results show that each of these two proteins accumulates in transgenic plants. GC-MS is being used to determine whether branched chain fatty acids or other novel compounds accumulate in these transgenic lines.

2. Isolation and Characterization of Proteins Associating with Calcium Oxalate Crystals.

Wyman, Aaron J. [1], Klanrit, Preekamol [1], Manalo, John P. M. [2], Ali, Ahson [3], Walker, Sheryl A. [1], Anderson, Carrie M. [1], Webb, Mary Alice [1].

1 - Purdue University, Botany and Plant Pathology, West Lafayette, IN, 47907,

2 - Wabash College, Biology, Crawfordsville, IN, 47933,

3 - Wabash College, Chemistry, Crawfordsville, IN, 47933,

The process of biomineralization has become increasingly important in fields ranging from materials sciences to medicine. However, many factors controlling biomineralization *in vivo* remain uncharacterized. The Webb lab is working on further elucidating calcium oxalate (CaOx) crystal formation in several eukaryotic systems, including plants. Plants [including grape (*V. labrusca*)] are hypothesized to form CaOx crystals for several reasons, including protection against herbivory, heavy metal detoxification, and as a mechanism to remove excess cytosolic calcium. Previous work on organisms that form calcium biominerals has implicated both inter and intra-crystalline proteins as having roles in controlling crystal growth and morphology. Similarly, the Webb lab has identified and investigated proteins associating with needle-shaped CaOx crystals (raphides) in grape. Among these raphide-associated proteins (RAPs) is a homolog of mammalian cochaperone Hsp-70 interacting protein (Hip). We present characterizations of the structure and function of several plant Hip orthologs. To identify more grape RAPs, we have utilized proteomics approaches. Both inter and intra-crystalline associating proteins were extracted, separated by SDS-PAGE, and analyzed by MALDI-MS to identify their peptide sequences. BLAST searches based on these amino acid sequences were conducted to identify possible eukaryotic homologs. We are cloning and expressing grape DNA sequences encoding for identified homologous proteins to investigate their polypeptides' effects on CaOx crystal development *in vitro*. It is hoped these findings will further elucidate CaOx formation *in vivo* and potentially aid development of methods for modifying the growth of human CaOx kidney stones.

3. Design, Assembly and Cleavage Characteristics of Zinc Finger Nucleases for Gene Targeting in Plants

Tovkach Andriy and Tzfira Tzvi

Department of Molecular, Cellular and Developmental Biology, University of Michigan, Ann Arbor, MI 48109

Double-strand breaks (DSBs) in plant genomes are typically repaired by the plant non-homologous end-joining (NHEJ) machinery, which usually leads to local mutagenesis due to small deletions at the repair site. Interestingly, artificial induction of DSBs by various restriction enzymes results in not only deletions, but also insertions of foreign DNA molecules into the repair site. This phenomenon could potentially be used for mutating specific sites in the plant genome and targeting foreign DNA molecules into them with zinc finger nucleases (ZFNs). ZFNs are a new type of artificial restriction enzymes which are custom-designed to recognize and cleave specific DNA sequences, producing DSBs. However, technical difficulties in the design, assembly and analysis of ZFNs have hindered the use of this new technology for plant gene targeting. We have recently designed a set of constructs and cloning, biochemical and *in-planta* analysis procedures for the newly designed ZFNs. Cloning begins with *de-novo* assembly of the DNA-binding regions of new ZFNs from overlapping oligos containing modified helices responsible for DNA triplet recognition, and their insertion between a nuclear localization signal and the *FokI* endonuclease domain. Following the transfer of fully assembled ZFNs into *E. coli* expression vectors, bacterial lysates were found to be most suitable for *in-vitro* digestion analysis of palindromic target sequences. An *in-planta* activity test was also developed to confirm the nucleic activity of ZFNs in plant cells. The assay is based on reconstruction of GUS expression following bombardment of a reporter and ZFN-expressing plasmids into mesophyll cells. Our new procedures, plasmids and assays bring us one step closer to efficient implementation of ZFN-based technology for gene targeting in plant species.

4. Transformation of cellobiohydrolase gene from *Trichoderma reesei* into tobacco and maize plants

Chuansheng Mei, Callista Ransom, Robab Sabzikar, Yanfen Zhai, Mariam Sticklen
Department of Crop and Soil Sciences, Michigan State University, E. Lansing, MI

Plant lignocellulosic biomass is renewable, cheap and globally available at 10–50 billion tons per year. At present, plant biomass is converted to fermentable sugars for the production of biofuels using pretreatment processes and addition of a mixture of cellulase enzymes [such as endo-1,4- β -glucanase (E.C. 3.2.1.4), exo-cellobiohydrolase (E.C. 3.2.1.91), and β -glucosidase (E.C. 3.2.1.21)]. Previously, we produced the *Acidothermus cellulolyticus* endoglucanase (E1) in maize and rice biomass, and confirmed that it could compete with the same enzyme produced in microbes. In order to effectively break down the cellulosic biomass into fermentable sugars, exoglucanase enzyme is needed, as it not only converts the xylose into pentose sugar, but it has synergistic action with the E1 enzyme. Our project focuses on production of a major exoglucanase, exo-cellobiohydrolase (CBH1), which specifically cleaves cellobiosyl units from the non-reducing end of cellulose polymer chains. We have made the construct containing the CBH1 gene from *Trichoderma reesei* under the control of the 35S promoter, tobacco mosaic virus translational enhancer, and the sequences for the targeting the gene product into the apoplast. Using the *Agrobacterium*-mediated method, we have transformed this exoglucanase into tobacco plants. PCR and Southern blot analysis have confirmed that the transgene integrated into the plant genome, and Northern blot analysis has shown that the transgene has been transcribed. Using particle bombardment, we have also transferred this gene into maize. Putative corn CBH1 transgenic plants have been obtained so far. The CBH1 activity in transgenic tobacco plants is 26.8 times higher than that of control plants. Also, the effects of the AFEX (Ammonia Fiber Explosion) pretreatment method on CBH1 activity will be evaluated in the process of cellulose to sugar conversion. Furthermore, the E1 transgenic maize will be cross bred with these CBH1 plants to obtain the synergistic effect on such conversion.

5. Investigation of the Expression and Localization of sHsp15 in *Arabidopsis thaliana*

Wojcik, Brandon¹ and Olsen, Laura¹ Department of Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, Michigan 48109, USA.

Defects in intracellular protein trafficking, specifically in peroxisomes, lead to many life-threatening diseases. These include Zellweger's syndrome, Alzheimer's, and X-linked adrenoleukodystrophy. An improved understanding of peroxisomal biogenesis will lead to more effective treatments. Many proteins destined for peroxisomes import via one of two signaling pathways, defined by targeting signal Type 1 and Type 2 (PTS1 and PTS2). This study will look at small heat shock protein 15 (sHsp15), which contains a putative Type 1 signal and may serve as an essential protein chaperone in the peroxisome. Cladograms will be constructed using bioinformatics tools to show the relationship between homologues of sHsp15 across other organisms. *In vitro* protein import assays will be used to affirm the import of sHsp15 into the peroxisome using the PTS1 pathway. Import efficiency will then be compared to glycolate oxidase, a known PTS1 protein, under varying cellular conditions. Finally, *in vivo studies*, using *Arabidopsis thaliana*, will attempt to determine sHsp15's function and localization in the cell. Function will be determined by taking phenotypic notes of mutant lines lacking the sHsp15 gene and monitoring mRNA expression using RT-PCR in plants under environmental perturbations. Localization will be determined using a green fluorescent protein tagged version of sHsp15. Results from this study could lead to a better understanding of peroxisomal processes, pose questions for future research in peroxisomal biogenesis, and contribute to the evolution of more efficient neurodegenerative disease therapies.

6. Four Conserved Cysteines and a Lysine Residue in the ATP Binding Domain are Important in the Function of Fea1, the Iron Transporter from *Chlamydomonas reinhardtii*

Moon, Hangsik¹, Rajamani, Sathish², Singh, Sareena¹, Siritunga, Dimuth³, Sayre, Richard¹.

¹Department of Plant Cellular and Molecular Biology, The Ohio State University, Columbus, OH 43210, ² Department of Plant Biology, University of California at Davis, Davis, CA 95616,

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Iron is an essential nutrient for virtually every organism because many cellular processes rely on proteins that incorporate iron-containing prosthetic groups. Although abundant in the soil, its bioavailability is low due to the relative insolubility of ferric ion (Fe³⁺).

A novel gene encoding an iron transporter, Fea1, was isolated from *Chlamydomonas reinhardtii* (Rubinelli et al., 2002). Recently, we have investigated the mechanism of iron transport by this unique protein. Comparative sequence analysis with the only two known proteins having similarity to Fea1 lead to the identification of four conserved cysteines and an ATP or GTP binding site known as P-loop. We proposed that the four cysteines may be involved in Fe coordination for uptake into the cell using ATP hydrolysis as the driving force. To test the hypothesis, each of the cysteines or a lysine residue in the ATP-binding domain was mutated to serine and arginine, respectively, and introduced into a yeast mutant that shows a limited growth in an iron-deficient medium. Unlike the WT Fea1, none of the mutant Fea1 could rescue the phenotype of the yeast mutant, demonstrating that those residues are critical for iron assimilation function. A model for Fea1 function will be presented.

7. Increasing the bio-available iron in cassava root by incorporating *Chlamydomonas FEAI* gene.

Uzoma Ihemere¹, Wai-Ting Chiu¹ and Richard T. Sayre¹.

¹Department of Plant Cellular and Molecular Biology, 318 W 12th Avenue, The Ohio State University, Columbus, OH 43210. (sayre.2@osu.edu).

Iron deficiency is a major problem in the developing countries of the world. The biggest problem associated with iron deficiency is anemia, which is associated with weakened immunity, mental retardation in children and increases in spontaneous abortions. We have studied the functions of the *Chlamydomonas FEAI* gene in *Arabidopsis thaliana*. Our studies show that the *Chlamydomonas FEAI* gene encodes a functional iron transporter in *Arabidopsis* as evidenced by the enhanced emergence of transgenic plants expressing the *FEAI* gene relative to the untransformed plants when grown under iron limiting conditions. In addition, the *Chlamydomonas FEAI* gene complemented the *irt1* mutant of *Arabidopsis* that lacks the ability to transport Fe. Recently, we have codon-optimized the *Chlamydomonas FEAI* gene for expression in cassava and transformed it into cassava cultivar TMS 60444 under the control of patatin promoter. Transformation was confirmed by PCR and RT-PCR analysis. The transformed plants had longer roots than the wild-type plants. The transformed cassava plants are currently undergoing Fe content analyses by ICP-MS to determine if the transformed cassava plants have higher Fe levels than wild-type cassava plants.

8. **Biomonitoring of Boron Micronutrient Stress in *Arabidopsis thaliana* and *Pelargonium X Hortorum*.**

Ying Deng ⁽¹⁾, Futong Yu ⁽¹⁾, Mei Chen ⁽²⁾, Jonathan Frantz ⁽³⁾, Scott Heckathorn ⁽²⁾, and John Gray⁽¹⁾. (1) Dept. of Biological Sciences, Univ. of Toledo, OH 43606, (2) Dept. of Earth, Ecological and Environmental Sciences, Univ. of Toledo, OH 43606, (3) U. S. Dept. Of Agriculture –Agricultural Research Services, Univ. of Toledo, OH 43606, email contact jgray5@uoft02.utoledo.edu

Horticultural growers typically rely on visual symptoms of nutrient deficiencies to guide them in spot treating their plants with appropriate fertilizers. Often, visible symptoms of nutrient deficiencies occur after it is too late to remedy the situation. There exists a period of nutrient stress before visible symptoms appear that is commonly referred to as “hidden hunger.” This refers to a period of time when the plant is altering gene and protein expression, such as for ion channels, transporters, leaf senescence, and reallocation of nutrients among a suite of other responses at the protein and genetic level. In this study we focused on the response of plants to deficient or toxic levels of the important plant micronutrient boron. In order to identify biomarkers linked to boron stress, we used genomics and proteomics approaches to monitor early responses of hydroponic *Arabidopsis* plants to high (1 or 3mM) or low (0.3 or 0mM) levels of boron. (For details of hydroponic system see poster by Futong Yu *et al.*, at this meeting). We report the isolation of candidate boron-response genes and proteins using microarray analysis and 2D gel electrophoresis/Mass Spectrometry respectively. The expression of candidate genes was validated, by profiling their expression using both quantitative real-time PCR and western blot analysis. We further report on the expression of a subset of boron-linked genes in the greenhouse crop *Pelargonium X Hortorum* (cv. Nittany Lion Red) under normal and boron stress conditions. In the long term, we will use this information to develop monitoring techniques or sentinel plants that greenhouse growers can use to for early detection of boron deficiencies.

9. **The COP9 signalsome is involved in ACR4 receptor turnover.**

Antony Chettoor, Kejian Li, Xueyuan Cao and Philip W.Becraft

Department of Genetics, Cell and Developmental Biology, Iowa State University, Ames, IA50010

The maize CRINKLY(CR4) gene encodes a receptor-like kinase that is involved in a array of developmental processes, including cell differentiation, cell proliferation, cell fate determination and pattern formation. ACR4 is believed to be the *Arabidopsis* CR4 orthologue with 60% amino acid identity and all the characteristic motifs of the maize CR4. A yeast two-hybrid screen was used to isolate putative downstream targets of ACR4. Six proteins that interact with the cytoplasmic domain of ACR4 were identified. They include the Cop9 Signalsome Subunits 5A and B (CSN5A, CSN5B), a putative protein phosphatase 2A regulatory subunit B delta (PP2A-B'□), a putative lipase and two leucine-rich repeat receptor like kinases (LRR-RLKs). Pull-down assays confirmed interactions between these proteins and ACR4 *in-vitro*. *In-vitro* kinase assays demonstrated that ACR4 could phosphorylate CSN5A, CSN5B, and the two RLKs, but the lipase and PP2A-B'□ were only weakly phosphorylated. *In-vivo* FRET experiments (Acceptor photobleaching method) demonstrated that the ACR4 receptor was in close proximity to CSN5A, CSN5B and PP2A within the context of a plant cell. Treatment of plants with Curcumin, a COP9 signalsome inhibitor, resulted in the accumulation of ACR4-GFP in plant cells. Genetic experiments to confirm the involvement of the Cop9 signalsome in the regulation of ACR4 are currently in progress.

10. Plant Infantry: *Arabidopsis thaliana* trichomes as defense organs

Manli Davis¹, Kengo Morohashi¹, Rebecca Lamb¹ and Erich Grotewold¹, (davis.2440@osu.edu)

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Control of gene transcription is of central importance in the development of any organism. Hierarchical arrangements (networks) of transcription factors (TFs) provide the information necessary to deploy genes with particular spatial and temporal patterns. It has been predicted that there are over 1,700 TFs encoded in the model plant species *Arabidopsis thaliana*. Many of these TFs are involved in various developmental processes but only a handful of them have known function. As part of our research on TFs, we are studying TFs involved in trichome initiation: *GLABROUS3 (GL3)/ENHANCER OF GLABROUS3 (EGL3)*, *GLABROUS1 (GL1)* and *TRANSPARENT TESTA GLABRA1 (TTG1)*. These genes encode bHLH, MYB and WD repeat proteins respectively; *gl3 egl3* double mutants and *gl1* or *ttg1* single mutants are glabrous. These proteins have been shown to form a transcriptional complex necessary for trichome initiation. The identification of the targets of these genes will provide information about the developmental processes of trichome development and the regulatory network involved in this process. As a complement to this avenue of investigation, we are also comparing gene expression between *gl3 egl3*, *gl1* and *ttg1* mutants and wild type in order to identify other trichome-enriched genes: 88 genes have significantly reduced expression in all three mutant backgrounds. Several of these genes had been previously identified as genes with high expression in trichomes. Identification of such genes will provide information on what function(s) the mature trichomes play in *Arabidopsis*. The proteins encoded by the trichome-enriched genes are involved in biotic and/or abiotic responses in plants, based on both bioinformatics and functional data. We conclude that *Arabidopsis* trichomes function as defense organs by serving as both a physical and a molecular barrier.

11. Function of Coactivator Proteins ADA2 and GCN5 in Cold Acclimation in *Arabidopsis*

Kanchan A. Pavangadkar^{1,2}, Michael F. Thomashow^{1,3,4} and Steven J. Triezenberg^{1,2,5}

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Covalent modifications of histones are important in regulating eukaryotic transcription. Lysine acetylation within the N-terminal tails of histones is associated with transcriptionally active genes and is catalyzed by histone acetyltransferases (HAT). GCN5 (a HAT) and ADA2 are components of coactivator complexes such as SAGA in yeast. The *Arabidopsis* genome encodes one homologue of GCN5 and two homologues of ADA2 (ADA2a and ADA2b). Null mutants of GCN5 and ADA2b have pleiotropic effects on plant growth and development whereas *ada2a* mutants show no aberrant phenotype.

Cold acclimation is the process by which plants increase their freezing tolerance upon exposure to low non-freezing temperatures. *Arabidopsis* ADA2 and GCN5 can physically interact with the transcriptional activator CBF1, which binds to and activates the expression of cold-regulated (*COR*) genes during cold acclimation. *ada2b* and *gcn5* mutants show a delay in activation and a reduction in expression of *COR* genes during cold acclimation. Chromatin immunoprecipitation (ChIP) assays showed that acetylation of histone H3 at the *COR* promoters increases upon cold acclimation. Plants overexpressing wildtype CBF1 showed increased H3 acetylation at *COR* gene promoters even without cold stress; a CBF mutant lacking the activation domain did not. Thus, CBF is sufficient to elicit an increase in H3 acetylation at the *COR* gene promoters. We hypothesized that CBF recruits GCN5 and ADA2b to acetylate histones at *COR* gene promoters and thus help activate *COR* genes. However, *ada2b* and *gcn5* mutants showed histone acetylation levels similar to wild type plants upon cold acclimation. Thus, ADA2b and GCN5 are not essential for cold-induced H3 acetylation at *COR* gene promoters.

12. Analysis of the KAT-1 promoter and NADP- Malic Enzyme in *Arabidopsis thaliana*

Patel, Lalita. Dept. of Biology, Eastern Michigan University

Plants that reduce water loss by transpiration pose less agricultural stress to the environment. Transpiration is limited by the size of pores, or stomata, on the surface of leaves and is related to the ion composition in surrounding guard cells (Blatt, 2000; Assmann, 2001). Potassium levels may be altered by the concentration of malate, a key counter-ion influencing guard cell pressure (Raschke, 1975; Zeiger, 1983, Outlaw and Zhang 2002). Levels of malate may be altered by the introduction of NADP-Malic Enzyme (NADP-ME) from maize (Laporte 2002). Transgenic *Arabidopsis thaliana* plants with increased NADP-ME expression are expected to have less open stomata, lose less water, and retain the ability to respond to changing environmental conditions (Laporte 2002).

In order to express NADP-ME strongly in *Arabidopsis thaliana* guard cells, I have analyzed and isolated a promoter of a guard cell specific potassium channel, KAT-1 (Nakamura et al, 1995). Analysis of the *Arabidopsis* genome was done to locate the promoter region. Custom primers were used to isolate the sequence from wildtype plants. PCR reactions under various conditions amplified the sequence, and were further amplified in transformed *E. coli*. The KAT-1 promoter will be inserted in *Agrobacterium* to then transform *Arabidopsis thaliana*. KAT-1 GUS transgenics will be grown to compare expression patterns. Expression of KAT-1 with NADP-ME in *Arabidopsis thaliana* should result in lower concentrations of malate in guard cells, and decreased aperture size of stomata.

13. Anti-sensing acyclic polyol (mannitol) biosynthesis in celery decreases salt tolerance

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Mannitol, a sugar alcohol that appears to serve as an osmoprotectant or compatible solute, is a major photosynthetic product in celery (*Apium graveolens* L.) where it is synthesized via the action of a NADPH dependent mannose-6-phosphate reductase (M6PR). Cytosolic localization of M6PR was first indicated by cellular fractionation, and this was confirmed by immunocytochemistry with light and electron microscopy, work that also indicated that M6PR occurs primarily in leaf mesophyll cells. *Arabidopsis* plants transgenic for the celery M6PR have since been shown to be salt tolerant. To confirm the abiotic stress effects of mannitol biosynthesis, we have transformed celery with an antisense construct of the celery leaf M6PR gene under control of the CaMV 35S promoter. Unlike wild type (WT) celery, independent antisense M6PR transformants did not accumulate significant amounts of mannitol in any tissue, with or without salt stress. In the absence of NaCl, and despite the lack of any significant accumulation of mannitol as the normally major photosynthetic product, antisense transformants were phenotypically and photosynthetically quite similar to the WT celery. However, in the presence of NaCl, mature antisense transgenic plants were significantly less salt tolerant, with reduced growth and photosynthetic rates, and some transformants were killed at 200 mM NaCl, a concentration that WT celery can ordinarily withstand. Although mannitol biosynthesis is enhanced in salt-treated WT celery, no such increase was observed in the anti-sense transformants. Like our previous 'gain of function' results showing enhanced salt tolerance in *Arabidopsis* plants transgenic for a sense M6PR construct, these 'loss of function' results in celery, with an antisense construct, demonstrate a major role for mannitol biosynthesis in developing salt tolerant plants.

14. Comparative functional analysis of plant pathogen responsive genes in model dicot and monocot pathosystems.

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Barley1 GeneChip expression profiling of barley (*Hordeum vulgare*) challenged with the powdery mildew fungus, *Blumeria graminis* f. sp. *hordei*, revealed over 200 genes that were up-regulated in incompatible responses but become suppressed following haustorial formation in compatible responses (Caldo *et al.*, 2004, 2006). These genes have been identified as candidates to mediate resistance to powdery mildew and are being analyzed for roles in barley defenses. To facilitate functional analyses of candidate genes and to investigate the broader importance of these genes in pathogen defense, we are also utilizing the interaction of *Arabidopsis* and *Pseudomonas syringae* pv. *tomato* (strain DC3000) as a model pathosystem. Greater than two-thirds of the barley genes of interest identify at least one homologous sequence in *Arabidopsis*. Further selection of *Arabidopsis* homologs for functional tests was influenced by phylogenetic analyses, gene-expression mining, gene ontology, and the availability of T-DNA lines and full-length cDNA constructs for facile generation of knockout, knockdown, or over expression lines. Genes predicted to act in signal transduction or be of unknown function were given a high priority. Loss-of-function T-DNA knockouts, constitutive over expression lines, or RNAi lines have been generated to several *Arabidopsis* homologs. The defense responses of these plant lines are quantified by bacterial growth in comparison to the corresponding wild type control plants. Alteration or knockout of genes involved in conserved defense responses is expected to result in a statistically significant difference in bacterial growth compared to wild type plants. Results forthcoming from pathogen testing will be presented at the conference.

15. Identification and characterization of the *Arabidopsis* mutant *scd2* (Susceptible to Coronatine-Deficient *Pseudomonas syringae* pv. *tomato* strain DC3118)

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Our group recently found that stomatal closure is involved in bacterium-induced innate immunity in *Arabidopsis*. *Arabidopsis* stomata actively close in response to the plant and human pathogenic bacteria, including *Pseudomonas syringae* pv. *tomato* (Pst) DC3000. In addition, PAMPs derived from bacteria, such as flagellin and lipopolysaccharides (LPS), also induce stomata closure. This stomata-based defense, however, could be overcome by coronatine, a phytotoxin virulence factor produced by Pst DC3000. A coronatine synthesis-defective mutant, Pst DC3118, is unable to inhibit stomatal closure and therefore does not efficiently enter *Arabidopsis* leaves through stomata to cause disease. However, Pst DC3118 is as virulent as the wild-type DC3000 when infiltrated into *Arabidopsis* leaves, bypassing the stomatal defense.

To identify the components involved in bacterium/PAMP-induced stomatal closure, a genetic screening was designed by taking advantage of the fact that the coronatine-deficient Pst DC3118 does not induce disease symptoms when applied on the leaf surface. An *Arabidopsis* T-DNA insertion population was screened for mutants that allowed Pst DC3118 to cause disease symptoms. One of the mutants identified is *scd2*. The stomata of this mutant plant showed only a partial response to ABA or flagellin²². Currently the *scd2* mutant is being analyzed for its basal resistance, gene for gene resistance, and HR responses.

In addition, we found that the wax composition of *scd2* leaves showed 80% decrease in alkane and two-fold increase in aldehyde. On the other hand, the wax composition in *scd2* stems remains similar to wild type plants. These results suggest that the *scd2* mutant is affected in leaf-specific wax biosynthesis.

Currently we are cloning the gene through physical mapping. Identification of the gene should improve our understanding of the connection between the leaf surface structure and plant defense against bacteria pathogens.

16. Identification of plant defense proteins that impair insect digestive physiology: Threonine deaminase as a case study

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In response to wounding or herbivore attack, plants synthesize various proteins that negatively affect the growth and development of arthropod herbivores. Some wound-inducible proteins, such as proteinase inhibitors (PIs), directly impair insect digestive physiology. Shotgun proteomic analysis has been used to identify proteins that accumulate in feces (frass) of *Manduca sexta* and *Trichoplusia ni* larvae reared on tomato (*Solanum lycopersicum*). This approach identified 13 distinct PIs, as well as several additional proteins that have a known or putative role in host plant defense. One of the most abundant proteins excreted in *M. sexta* and *T. ni* frass was a jasmonate-inducible isoform of threonine deaminase (TD2). TD2 contains an N-terminal catalytic domain and a C-terminal regulatory domain that is subject to feedback inhibition upon binding of isoleucine. We found that TD2 excreted in insect frass lacks the C-terminal regulatory domain and thus is insensitive to inhibition by isoleucine. The remaining catalytic domain was thermostable and active in an alkaline pH range of the lepidopteran gut. These properties of excreted TD are consistent with a role in degrading an essential nutrient (i.e., threonine) in the extreme environment of the lepidopteran gut. Molecular analyses provided evidence that tomato uses two different TD isozymes. TD2 is involved in threonine degradation in the insect midgut, while a second isoform TD1 functions in the biosynthesis of isoleucine.

17. Potential Role of Auxin Response Factor 9 in Gravity Signal Transduction

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Gravity response in plants involves three basic steps; signal perception, signal transduction and differential growth. Studies involving the gravity persistence signal (gps) mutants (Wyatt et al. 2002) have implicated the Auxin Response Factor (ARF) gene family in gravitropic signal transduction; in addition several ARF mutants have gravitropic phenotypes. The goal of this research was to determine if Auxin Response Factor 9 (ARF) is involved in the plants response to gravity. Wild type Arabidopsis were exposed to one hour gravity at 4°C and returned to vertical at room temperature (GPS treatment) as described in Wyatt et al. 2002, quantitative RT-PCR of the ARF9 transcript was performed at several time points during this treatment. Interestingly, ARF9 transcript was increased after the GPS treatment. In addition, T- DNA insertion mutants for ARF9 were obtained from the SALK Institute and screened for a gravity response using the GPS treatment. One line, with the T-DNA insertion located in exon 12 near the C terminus of ARF9, displayed increased gravitropic curvature after the GPS treatment indicating that ARF9 is involved in gravitropic signal transduction.

(Partially Supported by Grasselli-Brown Undergraduate Research Award, Ohio University, to DRR and NASA: NAG2-1608 to SEW.)

18. The role of phytochrome C in gravitropism and phototropism

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Light and gravity are among the most important environmental stimuli that influence plant form and development. Plants sense light using the red-light-absorbing phytochromes and the blue-light-absorbing cryptochromes and phototropins. The phytochromes, which are molecular family consisting of PHYA–E, have been shown to be involved in both phototropism and gravitropism. Phytochrome C is considered one of the more minor forms, and very little is known about the role of PHYC in plant development. In this project, we studied the role of PHYC in growth and tropisms by using the mutants *phyCD* and *phyD* and comparing them to wild-type Wassilewskija (WS) plants. Gravitropism and blue-light-induced phototropism were examined in young seedlings and in mature plants. The most significant differences were found in experiments with inflorescence stems, and PHYC modulated both gravitropism and phototropism in these organs. Phytochromes play an important role in helping plants sort through the constant bombardment from environmental stimuli and in determining the final growth form of the plant. (Supported by Miami University Undergraduate Summer Scholar Program).

19. Arabidopsis *XT1* and *XT2* encode xylosyltransferases involved in xyloglucan biosynthesis

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Xyloglucan (XyG) is the major hemicellulose in the primary cell walls of dicots and nongraminaceous monocots. XyG is thought to act as a cross-linking glycan between adjacent cellulose microfibrils to form a three dimensional cellulose-xyloglucan network that functions as the principal load-bearing structure of the primary cell wall. Recently, seven candidate XyG xylosyltransferase (XT) genes were identified in *Arabidopsis* (Faik et al., 2002). Heterologous expression of the candidate *Arabidopsis* XyG XT genes in yeast and insect cells demonstrates that *XT1* (At3g62720) and *XT2* (At4g02500) encode proteins with α -xylosyltransferase activity that is capable of catalyzing the addition of multiple α -(1,6)-xylosyl residues to either cellopentaose or celohexaose acceptor substrates (Faik et al., 2002; Cavalier and Keegstra, 2006). To determine if *XT1* or *XT2* is involved in XyG biosynthesis, reverse genetics studies were employed in which *Arabidopsis xt1*, *xt2*, and *xt1/xt2* T-DNA insertion lines were developed and characterized. The *xt1* and *xt2* single knockout lines produced normal plants with a weak root hair phenotype, while the *xt1/xt2* double knockout line produced plants that developed slower, were slightly smaller, and had a severe root hair phenotype. Furthermore, there was a significant decrease in the modulus of elasticity and ultimate strength of the *xt1/xt2* mutant cell wall with respect to Columbia wild-type. While biochemical analysis of the *xt1* and *xt2* single knockout lines indicated that they contained XyG that was indistinguishable from the Columbia wild-type, biochemical analysis demonstrated that 7 day-old *xt1/xt2* seedlings: i.) lacked XXXG, XXLG, XLXG, and XLLG XyG oligosaccharides in either EGII or XEG digested cell wall material; ii.) lacked the diagnostic XyG disaccharide isoprimeverose in driselase-digested cell wall preparations; and iii.) had a significant decrease in glycosyl linkages that correspond to XyG. By combining heterologous expression studies and reverse genetic studies, we are able to conclude that *XT1* and *XT2* encode xylosyltransferases that are involved in XyG biosynthesis. What remains to be clarified is how the *xt1/xt2* mutant *Arabidopsis* plants are able to grow and develop relatively normally with so little XyG.

20. Functional characterization of Lysine-rich AGPs by reverse genetics

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Arabinogalactan proteins (AGPs) are a class of hydroxyproline-rich glycoproteins found at the plant cell surface. They are highly glycosylated and are thought to play important roles in plant cell differentiation, cell-cell recognition, embryogenesis and programmed cell death. There are three homologous genes in the lysine-rich AGP subfamily in Arabidopsis : AtAGP17, AtAGP18 and AtAGP19. They are composed of an N-terminal signal peptide, a Pro/Hyp-rich central domain disrupted by a lysine-rich region and a C-terminal GPI anchor addition region.

A T-DNA knockout mutation in AtAGP19, was obtained and examined. Compared to wild type plants, the *atagp19* mutant had: 1) lighter green leaves containing less chlorophyll and anthocyanins, 2) rounder leaves, with shorter petioles, 3) shorter and thinner inflorescence stems, 4) slower growth with delayed and reduced flowering, 5) fewer siliques and seeds and 6) fewer lateral roots. Complementation of this mutant with the wild type AtAGP19 gene restored the wild type phenotypes. How a mutation in AtAGP19 can elicit these phenotypic changes is still unknown. In order to begin to address this question, a microarray approach was used to elucidate changes in gene expression associated with the *atagp19* mutant in Arabidopsis leaves. Wild type plants were grown together with homozygous *atagp19* mutant plants under identical environmental conditions for 14 days. Total RNAs were extracted from wild type and *atagp19* mutant leaves using the Qiagen RNeasy Plant Mini Kit and sent to the University of California, Irvine UCI DNA & Protein MicroArray Facility for microarray analysis. RNA expression levels for the ~22,000 genes in both wild type and *atagp19* mutant plants were analyzed by ArrayAssist 3.0 (Stratagene). Among these genes, 79 genes are up-regulated more than two-fold and 32 genes are down-regulated more than two-fold. These gene products are predicted to be in the chloroplast, endomembrane system, mitochondria, nucleus, peroxisome and microtubule. Expression levels for several of these genes were further examined by QPCR to confirm the microarray data.

An overexpression approach was also used to elucidate AtAGP17/18/19 function(s). Constructs with EGFP (Enhanced Green Fluorescent Protein)-AtAGP17/18/19 fusion protein under the control of the CaMV35S promoter were introduced into Arabidopsis using Agrobacterium-mediated transformation. T1 seeds were screened on kanamycin-selective media and PCR with vector-specific primers was performed to detect the incorporation of the constructs into the plant genome. T2 plants were used for phenotype analysis. Compared to wild type, transformants which overexpressed AtAGP18 were significantly shorter and highly branched. Construct with only EGFP was also introduced into Arabidopsis as a control and T1 seeds are being screened now.

21. Evolutionary Mechanisms of Prezygotic Isolation in Two Replicate Sublineages of Hawaiian Violets (*Viola*, Violaceae).

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On the Hawaiian Islands, two replicate sublineages of Violets, containing montane bog and swamp forest ecomorphs, are distributed on the islands of Kauai (*V. wailenalenae* and *V. kauaensis*) and Molokai (*V. robusta* and *V. maviensis*). The two sublineages represent evolutionary replicates, separated by approximately 2 million years, and offer a unique opportunity to study the evolution of prezygotic isolation. During the summers of 2005 and 2006, the temporal, ethological, and putative mechanical isolation mechanisms between intransland taxa were documented. On Kauai, observations of flowering phenology during the months of July and April revealed that *V. kauaensis* produces cleistogamous flowers while *V. wailenalenae* produces only chasmogamous flowers. On Molokai, *V. robusta* and *V. maviensis* produce chasmogamous flowers concurrently. On both Kauai and Molokai, no Violet species are visited by insect

pollinators. Pollinator exclusion experiments indicate that each species is capable of producing fruit in the absence of insect pollinators. Anatomical investigations of flowers that developed inside pollinator exclusion bags show that the Hawaiian Violets are capable of producing fruit autogamously. On Molokai, a hybrid, *V. x luciae* (*V. maviensis* x *V. robusta*) is distributed in the ecotone between the bog and swamp forest habitat. The hybrid has 37% pollen stainability, indicating that it has substantially depressed fertility, and possesses malformed carpels (probably therefore an F1). The presence of the hybrid, along with the putative lack of temporal isolation on Molokai, indicate that prezygotic isolation mechanisms on this island are not as fully developed as on Kauai. Because no differences were seen in the other prezygotic isolation mechanisms studied (ethological and mechanical), temporal isolation may represent the last step in the establishment of complete prezygotic isolation mechanisms among the Hawaiian Violets.

22. The Evolution of Young Gene Duplicates in *Arabidopsis thaliana*.

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Before contributing to genome and organismal evolution, duplicate genes must become established or *fixed* in the population. We are interested in how recent gene duplicates become fixed in the genome of *Arabidopsis thaliana*. Specifically, does selection or drift drive the fixation of duplicate genes? In order to address this question, we determined the frequency of duplicate loci in a sample of *A. thaliana* accessions and assessed whether they conformed to the drift expectation for a neutral allele segregating in a population given their age. Before using laboratory techniques, we identified a list of duplicate loci in the *Arabidopsis* genome with low levels of sequence divergence, a characteristic of recent gene duplicates. We used the genomic resources on TAIR (The Arabidopsis Information Resource) to validate these duplications as well as the extent of each duplication. There was a trend in the size of the duplications; duplications involved relatively small regions of sequence and included very little of the promoter region. By aligning the duplicated regions, we were able to identify polymorphic sites which we used to design CAPS (Cleaved Amplified Polymorphic Sequence) markers specific to each duplicate. Using PCR we were able to differentiate specific duplicate genes in the genomes of a sampling of *A. thaliana* accessions and estimate the frequency of the duplicate in the population. Contrary to previous findings, it appears that random drift may be the predominant force acting on the fixation of duplicate loci.

23. Two putative Poly (ADP-ribose) polymerases, RCD1 and SRO1, play important, partially redundant roles in *Arabidopsis* development

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RADICAL-INDUCED CELL DEATH1 (RCD1) has been identified as a stress response gene, interacting with several stress and hormone response pathways. RCD1 consists of a poly(ADP-ribose) polymerase (PARP) domain and a WWE protein-protein interaction domain. PARPs mediate attachment of ADP-ribose units from donor NAD⁺ molecules to target proteins and have been implicated in a number of processes including DNA repair, apoptosis, transcription, and chromatin remodeling. RCD1 and SIMILAR TO RCD ONE1 (SRO1) are the only two proteins encoded in the *Arabidopsis* genome containing both WWE and PARP domains; similar proteins have been found in all groups of eukaryotes. Like *RCD1*, *SRO1* is expressed in all plant tissues examined. We have isolated homozygous null mutants in both *RCD1* and *SRO1*, *rcd1-3* and *sro1-1*, respectively. *rcd1-3* plants display similar phenotypic defects to those reported for previously isolated alleles (*rcd1-1* and *rcd1-2*), like reduced stature, malformed leaves and early

flowering. In addition, the plants have abnormal phyllotaxy, small, deformed floral organs, increased lateral root number and length, and shorter primary root. *sro1-1* plants display some subtle developmental defects similar to the root and flower phenotypes of *rcd1-3*. However, plant height, leaf shape and size, and flowering time appear normal. Preliminary analysis of stress response indicates that *SRO1* may have a different function than *RCD1* in these pathways. Double mutant plants of *rcd1-3;sro1-1* display severe developmental defects including germination defects, extreme dwarfism, abnormal flowers, and short siliques. The double mutant phenotype suggests that *RCD1* and *SRO1* are at least partially redundant with one another and that they are essential genes for plant development.

24. CHARACTERIZATION OF PROTEINS FROM CORN COLEOPTILE EPIDERMIS THAT ARE INVOLVED IN AUXIN-INDUCED GROWTH

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Rapidly growing corn coleoptiles display a phenomenon called “tissue tension”. When they are cut longitudinally, they curve outwards with the epidermis on the concave side of the section. Tissue tension has been interpreted as the manifestation of two conflicting forces: the epidermis is under tension because it is growth limiting, while the inner tissue does not limit growth and is under compression. During examination of the cell ultrastructure of rapidly growing plants, osmiophilic particles (OPs) had been observed in several plant species. These particles are 80-300nm in diameter. Electron microscopy and labeling experiments had shown that they are closely associated with the outer epidermis of growing tissues, are going through the secretory pathway, and are, at least in part, proteinaceous. From their location and time of appearance we can assume that they are related to either cell-wall or cuticle biosynthesis. As precursors of the plant cuticle, they would be essential in multiple ways: in addition to playing a role in plant growth, they may be important in the defense against pathogens and in the prevention of water loss. We used a proteomics approach to try to identify novel proteins involved in regulating plant growth via cell wall or cuticle biosynthesis by comparing the protein profile of slow versus rapidly growing coleoptile and coleoptile epidermis.

We were able to identify over 80 proteins that appear to be induced in rapidly growing coleoptile epidermis. Half of those are related to protein synthesis/maintenance and 11% are potentially associated with the cell wall, cuticle, or lipid metabolism. We are now analyzing the expression and distribution of the latter proteins plus an additional three hypothetical proteins with unknown function.

25. Identification and characterization of floral genes in a cleistogamous species *Viola pubescens* (Violaceae)

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Many plants, including most species in the genus *Viola* (commonly known as violets), can produce both open (chasmogamous) and closed (cleistogamous) flowers on the same plant. Chasmogamous and cleistogamous flowers produce seeds by outcrossing and selfing respectively, and the mixed breeding system is considered a successful reproductive strategy. But the underlying molecular basis of the floral dimorphism is not known. Gibberellic acid (GA, a plant growth hormone) functions in triggering flowering and has been suggested to play some roles in the floral dimorphism. The *LEAFY* (*LFY*) gene of *Arabidopsis* and its orthologs in other plants are responsible for the initiation of floral meristems and the regulation of the downstream flower development. Afterward, the ABCE classes of floral organ identity genes collaborate to give rise to a flower. The GA 20-oxidase (a key enzyme in GA biosynthesis), *LFY* and

most of the ABCD floral gene orthologs of a widespread North American violet, *V. pubescens*, were identified by polymerase chain reaction (PCR). Semi-quantitative reverse transcriptase-PCR indicated that all the genes were expressed in both types of flowers of *V. pubescens*, but the expression levels showed significant differences. The *VGA 20-oxidase* was expressed more in chasmogamous flowers than in cleistogamous flowers. At least four *VLFY* gene transcripts were detected, and different transcripts displayed different expression patterns between the two types of flowers. The A class genes were expressed equally in both types of flowers. Expression of B class genes was increased in chasmogamous flowers as compared to cleistogamous flowers, while the C class genes' expression was much more reduced in chasmogamous flowers than in cleistogamous flowers. Overall, the different expression patterns of the floral genes explained the morphological differences between the two types of flowers. Our study provided the first step to understanding the molecular control of the floral dimorphism. It also lays the basis for further research such as the environmental and molecular regulations of the violet floral genes.

26. Identification of SEPALLATA Floral Gene Orthologs in *Viola pubescens*

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The SEPALLATA (*SEP*) gene family represents E-class genes in the ABCDE flower model and comprises two gene clades and three subclades. While not exclusively involved in flower development, *SEP* regulates the growth of petals, stamens, and carpels in association with ABC floral genes. The ABCDE model for the genetic regulation of flower development describes chasmogamous flower development but currently, the role of floral genes in cleistogamous flower development remains unknown. This project sought to discover *SEP*-like genes in the cleistogamous herb *Viola pubescens*. With primers based on the *SEP* sequences of *Arabidopsis thaliana*, polymerase chain reaction (PCR) amplified suspected *SEP*-like genes. Cloning and sequencing of PCR products uncovered the existence of one *SEP3*-like gene as well as one *SEP1/2*-like gene representing a major gene clade and subclade respectively. The presence of *SEP*-like genes in *V. pubescens* was expected and supports the importance of *SEP* in floral development. To date, nothing is known about the involvement of *SEP* in mixed-breeding systems as exemplified by *V. pubescens*. A more complete understanding of the genetic regulation of cleistogamous flowers may provide unique solutions to the threat of transgenic DNA escaping into the wild.

27. Autophagy Protein 6 (ATG6) is required for post-microsporogenesis pollen development in *Arabidopsis thaliana*

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Autophagy is an inducible, intracellular recycling pathway that extends the life of an organism in hostile conditions. Under low sugar or nitrogen regimes, autophagy allows for sequestration of cytoplasmic components into vesicles that traffic to the vacuole for degradation. AtATG6, is a homologue of mammalian Beclin 1 and yeast Atg6p/Vps30p. Screening of *atg6* tDNA-insertional lines indicates that AtATG6 is an essential protein as the homozygous state is lethal. In addition, heterozygous plants displayed variable growth phenotypes in comparison to wild-type plants. Progeny segregation of 50:50, wild-type to heterozygous plants, and the lack of aborted seeds in siliques on heterozygous plants, suggest a germ cell defect. Pollen from *atg6* heterozygotes was crossed into *glabra-2* plants and yielded only wild-type progeny whereas progeny of the reciprocal cross yielded the expected ratio. This indicates a pollen defect is responsible for loss of homozygote segregants. PCR performed on pollen from *atg6* heterozygote plants revealed the TDNA is present in the mature pollen population, suggesting the phenotypic defect is post-microsporogenesis. Furthermore plants homozygous for *qrt-1(-/-)* and heterozygous for *atg6* produced

tetrads that were trinucleate and stained uniformly with Alexander stain. However, *qrt-1(-)/atg6(+/-)* pollen exhibited different germination efficiencies *in vitro*. Finally a series of *in vivo* pollen tube guidance experiments revealed no defects in pollen tube growth or guidance. While it is yet to be determined whether ATG6 acts in an autophagy- dependant or independent manner during pollen development, this data suggests novel connections between plant stress responses and reproductive biology.

28. Functional Genomic Analysis of Tomato (*Solanum*) Trichomes

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Trichomes are epidermal protuberances present on various tissues of many plants. Typically, trichomes are classified as being either non-glandular or glandular with the latter capable of synthesizing and secreting a variety of phytochemicals. Because of the location on plant surfaces of trichomes, their physical presence and the metabolites they produce can function in response to a number of environmental stresses including protection against herbivore and pathogen attack. Our interest is in understanding what types of chemicals are present and how they are produced in glandular trichomes of tomato and its closely related wild species of the genus *Solanum*. We are using a combination of metabolic profiling, EST sequencing, and proteomics for discovery of novel genes influencing trichome chemistry. Trichome metabolites are profiled using GC-MS, LC-MS, and high-throughput flow-injection electrospray ionization MS. Deep-sequencing of isolated trichome cDNAs using massively parallel pyrosequencing together with shotgun proteomics of trichome protein is being used to identify candidate genes controlling trichome metabolism. Results from metabolic profiling together with EST sequencing and proteomics using isolated trichomes from *Solanum lycopersicum* cv. M82 will be presented.

29. Cyanide metabolism, protein production and post-harvest physiological deterioration in cassava

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Cyanide in cassava is released via the breakdown of the cyanogenic glucoside, linamarin, via a two step process, beginning with the removal of the glucoside followed by spontaneous or enzymatic decomposition to release HCN and acetone. The enzyme β -cyanoalanine synthase (β -CAS) can convert the released HCN and cysteine to β -cyanoalanine (Blumenthal et al., 1968), which is subsequently converted to asparagine (by a hydratase), and finally to aspartate and ammonia (by a nitrilase) (Siritunga and Sayre, 2003). We are investigating the possible role of cyanide as a primary metabolite in protein production and as a source of reduced nitrogen. Evidence for the possible role of cyanide as a primary metabolite comes from the fact that transgenic low cyanide plants only grow in tissue, where reduced nitrogen is supplemented in growth medium as ammonia (Siritunga and Sayre, 2004). We have determined that the activity of cyanide assimilation enzymes in roots is substantially greater than that in leaves. In addition, we have shown that cyanide detoxification pathways leading to the production of thiocyanate do not compete with cyanide assimilation pathways in roots. Preliminary evidence also supports a link between cyanogenesis and post harvest physiological deterioration (PPD), a process which occurs

in cassava within 72 hours of harvest. It has recently been demonstrated that PPD is associated with reactive oxygen species (ROS) production in cassava roots (Reilly et al., 2003). We show that transgenic low cyanide plants have reduced ROS production compared to wild-type plants with normal cyanide levels. In addition, we have shown that addition of cysteine, one of the substrates of β -CAS to roots sections reduces PPD presumably associated with cyanide assimilation and a reduction in poisoning of cytochrome C oxidase which leads to ROS production. We also discuss our current strategies in engineering the cyanide assimilatory pathway for enhanced amino acid pools in cassava roots.

30. Cytoplasmic and vacuolar expression of linamarase in cassava roots for protein content enhancement

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Cassava is a shrubby tropical perennial plant. Its roots are in particular favored for consumption and have become over the past 30 years a staple food for millions of people in Sub-Saharan Africa. (FAOSTAT, 2006) Cassava roots however pose the nutritional constraint of having low protein content, as well as potentially toxic levels of the cyanogenic glycoside, linamarin. Linamarin synthesis takes place in the leaves, from there it is symplastically transported to the roots and stored. The cyanogenic glycoside's role was thought to be solely that of herbivore deterrent but it has been found that it also plays a role in nitrogen metabolism, as transgenic cassava plants with reduced linamarin synthesis need to be supplemented with ammonia to achieve adequate growth. (Siritunga et al., 2004) Linamarase is the enzyme that catabolizes the deglycosylation of linamarin, yielding acetone cyanohydrin, which can degrade to produce cyanide. This enzyme is localized to the cell wall (Mkpong et al, 1990), consequently the degradation of linamarin and the release of cyanide occurs only after cell rupture.

We propose that by expressing linamarase in the cytoplasm or the vacuole the deglycosylation of linamarin will occur during plant growth providing a reduced nitrogen source for assimilation in to amino acids. Two vectors have been designed for this purpose, for cytoplasmic expression the signal peptide has been eliminated from the sequence of linamarin and for vacuolar targeting a C-terminus Vacuolar Targeting Domain was added. To date, we have generated several putative transformants with these vector and one confirmed transgenic cassava plant expressing linamarase in the vacuole. A model will be described for nitrogen assimilation via cyanide.

31. A Plastid Polyribonucleotide Phosphorylase Suppresses Variegation in the Arabidopsis *var2* Mutant

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The Arabidopsis *var2* variegation mutant defines a nuclear gene for a chloroplast FtsH metalloprotease. Leaf variegation is expressed in homozygous recessive individuals of the mutant. The cells in the green leaf sectors of *var2* contain morphologically normal chloroplasts, whereas cells in the white sectors contain abnormal plastids lacking organized lamellar structures. *var2* mutants are hypersusceptible to photoinhibition, and consistent with this phenotype, VAR2 has been shown to be involved in the D1 repair cycle of photosystem II, likely by affecting turnover of the photodamaged D1 polypeptide. A second-site suppressor screen of *var2* yielded several lines in which the variegation phenotype of *var2* is significantly modified. Some of these lines have a "central yellow" (CY) phenotype, in which the younger leaves on the

rosette are pale-green or yellow, then turn fully-green as they develop and expand. One suppressor line with a CY phenotype, 2484, was chosen for further analysis. Map-based cloning revealed that the suppressor gene in 2484 codes for a plastid Polyribonucleotide Phosphorylase, which involved in rRNA processing and chloroplast protein translation. We designated this gene *CY1*. Isolation of the *cy1* single mutant showed the same phenotype as the double mutant, which demonstrates that *cy1* is epistatic to *var2*. Our results suggest that VAR2 and CY1 act antagonistically in chloroplast biogenesis, and that downregulation of CY1 lowers the requirement for VAR2 in plastid development. The isolation of a *cy1* mutant represents an important advance in the generation of tools to understand variegation mechanisms and photoprotection in plants.

32. Identification and characterization of Mep1, a novel plastid envelope protein

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The plastid envelope membranes represent the interface between the metabolic networks of the cytosol and the plastid. Yet to date only a few metabolite transport proteins have been characterized at the molecular level. We hypothesized that a proteome analysis of *Zea mays* (maize) mesophyll plastids will generate candidate proteins that catalyze the metabolite fluxes that are enhanced in C4 plastids of *Z. mays* compared to previously analyzed other plastid types. A qualitative and semi-quantitative analysis based on peptide counts of the proteome of *Z. mays* mesophyll plastid envelopes will be presented. We identified proteins in a dynamic range of 300-fold, from extremely abundant metabolite transporters to very low abundant proteins, likely involved in signaling. We compared this proteome to previously published proteomes.

We hypothesize that transport proteins which characterize the metabolite fluxes required for C4 metabolism in *Z. mays* are abundant. Here, we present the analysis of Mep1 (**M**esophyll **e**nvelope **p**rotein **1**), one of the most abundant proteins in maize mesophyll plastid envelopes. The knock-out of the corresponding gene in *Arabidopsis thaliana* bleaches upon exposure to high light intensities or during 24h days. This phenotype is cured when the plants are grown in elevated CO₂ concentrations or low light conditions. We confirmed the plastid localization with C-terminal GFP fusions and determined the expression pattern with promoter::GUS fusions. A target metabolite analysis reveals the block of metabolism within the plants and suggests a possible role for Mep1 as the glycerate/glycolate transporter in photorespiration in *A. thaliana*.

Currently we are analyzing the transport capacities of Mep1 in whole plastids. Isolated plastids from *A. thaliana* wild type and knock-plants and *Zea mays* mesophyll tissue are fed with candidate substrates and uptake is monitored with a Clark-type oxygen electrode. These results together with the biochemical characterization of the heterologously expressed proteins will ultimately reveal the transport capacities of Mep1 in *A. thaliana* and *Z. mays*.

33. TGD3, an ATPase Protein of Arabidopsis, Functions in ER-to-Plastid Lipid Trafficking

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Membrane lipid transfer between subcellular membranes is essential for the growth and maintenance of all cells. Arabidopsis mutants that are deficient in the ER-derived galactolipid biosynthesis pathway provide new insights in understanding the lipid transfer phenomena between these membranes. Galactolipids in

these mutants are derived primarily from the plastid pathway. The name *tgd* represents their complex lipid phenotype, including the accumulation of triacylglycerols (TAG) and trigalactosyldiacylglycerol (TGDG) in leaves. Two genes identified from two of these mutants, *TGD1* and *TGD2*, encode the permease and substrate binding protein, respectively, of a putative lipid transporter at the inner chloroplast envelope membrane. TGD3, an ATPase protein of Arabidopsis, is hypothesized to be the third component of this transporter. Similar to the *tgd1* and *tgd2* mutants, TAG and TGDG also accumulate in a *tgd3* mutant carrying a T-DNA insertion in the promoter region of the TGD3 ORF. The TGD3 protein has basal ATPase activity *in vitro* and is localized on the inside of the inner chloroplast envelope membrane. Protein orthologs of TGD1-3 proteins are found in all bacteria and the respective genes are organized in operons, suggesting a common role of these proteins. Based on current evidence, it is hypothesized that TGD3 is the ATPase component of a bacterial-type ATP binding cassette (ABC) transporter involving TGD1 and TGD2 that functions in polar lipid trafficking from the ER to the plastid. Given the accumulation of phosphatic acid (PA) in the *tgd1* mutant and the substrate binding specificity of the TGD2 protein, it is likely that PA is the substrate transported.

34. Forisomes – A Unique ATP-Independent Contractile Apparatus in the Sieve Tubes of the Legumes

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Technically speaking, the phloem of higher plants is a microfluidics system that enables the distribution of photoassimilates throughout the plant body. The legume family possess a unique mechanism for phloem flux regulation (Knoblauch and Peters, 2004). Elongate protein bodies, which we have called forisomes (*gate-bodies*), block individual sieve tubes in response to increased cytosolic Ca^{2+} (Knoblauch et al, 2001). To do this, they contract anisotropically and increase their volume up to nine-fold. This Ca^{2+} -driven process is independent of ATP (Knoblauch et al. 2003). It can be completed within less than one second, is fully reversible by removal of Ca^{2+} , and can be induced electrically *in vitro*.

We here review our most recent, unpublished results: 1, forisome contraction probably involves the reversible establishment of highly ordered macromolecular arrays, as visualized by polarization microscopy. 2, studies using high-speed photography of contracting forisomes appear to suggest that the longitudinal and radial components of the contraction/expansion reactions proceed largely independent of each other. 3, investigations into the taxonomic distribution of forisomes suggest that forisome contractility in fact is a unifying and defining trait of the faboid legumes, but that this trait has been lost at least once in the radiation of this subfamily.

35. Integration of *Agrobacterium*'s T-DNA Molecules into Genomic Double Strand Breaks

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The mechanism of T-DNA integration in plant cells remains largely unexplored even though several different T-DNA integration models have been suggested. Recent genetic and functional studies have revealed the importance of host proteins involved in DNA repair and maintenance via T-DNA integration. This suggests a possible route for T-DNA integration into the double strand breaks (DSB) by non homologous end joining (NHEJ) pathway. We have previously shown that double stranded T-DNA molecules preferentially integrate into a DSB in transgenic plants carrying an I-*SceI* endonuclease recognition site which, upon cleavage with I-*SceI*, generates a DSB. This process is limited by its ability to

induce only a single break in the host genome. To overcome this hurdle, we considered using a different enzyme, with natural recognition sites in the host genome. Bioinformatics analysis revealed that the *AscI*, an 8 base cutter recognizes ca. 80 sites in the *Arabidopsis* genome, making it an excellent choice for induction of multiple DSBs. Since *AscI* is of prokaryotic origin, we first tagged it with a plant nuclear localization signal to achieve its efficient localization to the plant cell nucleus. We then developed a system to test the *AscI* restriction ability *in-planta*, by restoration of transient GUS expression of a modified plasmid and the *AscI* enzyme in *Arabidopsis* leaves. Using bioinformatics, we designed a unique amplification restriction pattern for all the *AscI* recognition sites on the *Arabidopsis* genome. This allows the identification of disrupted *AscI* recognition sites and site-specific integration of T-DNA molecules, following a transient expression of *AscI* in plants. We discovered that DSBs acts as attractants of T-DNA molecules and we are currently exploring the significance of such breaks to the integration process.

36. Optimization of water-use efficiency by an endophytic fungus in *Lolium arundinaceum* grasses

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Neotyphodium coenophialum (Ascomycota: Clavicipitaceae) grows intercellularly in above-ground parts of C₃ grasses. It is an asexual fungus that is transmitted through seed of its host plants. This grass/endophyte association is based on the protection of the host from herbivory and improved drought stress. We performed experiments to determine if a change in stomatal conductance impacts the instantaneous water-use efficiency (WUE), according to the stomatal optimization theory, in endophytic-infected (E+) versus uninfected (E-) *Lolium arundinaceum* grasses grown in controlled environmental chambers over 10-week periods. Grasses were cut at 6 weeks after germination and allowed to regrow under high and low soil moisture availability. Soil moisture was allowed to decline after seven weeks in the low water treatment until severe stress was demonstrated. We found no difference in WUE among E+ and E- plants when water was not limiting. Uninfected plants showed a significant decline in WUE and an increase in ratios of internal to external leaf CO₂ partial pressure (p_i/p_a) when stomatal conductance decreased under severe drought stress. E+ plants maintained a constant WUE and p_i/p_a across a wide range of stomatal conductances in compliance with the optimization theory. The endophyte clearly maintains a constancy of water-use efficiency in the grass under drought conditions.

Additional Registered Poster Presentations

37. Evidence for Novel Regulatory Pathways that Contribute to Cold Acclimation in *Arabidopsis*.

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The CBF/DREB1 family of transcriptional activators plays a major role in cold acclimation, the process whereby certain plants increase in freezing tolerance in response to low non-freezing temperatures. Analysis of plants overexpressing CBF1, 2, or 3 indicates that the CBF pathway regulates expression of approximately 15% of cold-responsive genes, the CBF regulon, which bring about an increase in freezing tolerance (1, 2, 3). Additionally, the metabolome of warm grown CBF overexpressing plants closely resembles that of cold-treated plants (3). However, transcriptional analysis of cold-treated plants (4) and analysis of several mutants including *esk1* (5), *ada2a* (6) and *hos 9* (7) has provided evidence for the existence of non-CBF pathways with roles in cold acclimation. Here we further explore the extent to which the CBF cold response pathway contributes to cold acclimation. *Arabidopsis* plants were transformed with a gene encoding a truncated version of the CBF2 protein, designated CBF2 Δ C, placed under control of the strong constitutive CaMV 35S promoter. The CBF2 Δ C protein contains the N-terminal amino acids,

including the AP2 DNA binding domain, but lacks the C-terminal region, which includes multiple activation domains (8). Transcriptional analysis of CBF2 Δ C overexpressing plants indicates that a majority of cold-responsive genes were significantly affected in CBF2 Δ C expressing plants compared to wild-type plants. Approximately 10% of the cold-regulated genes were found to be strongly dependent on the CBF pathway (reduced 90% or more in CBF2 Δ C plants compared to wild type). However, even though there was an effect on many cold-regulated transcripts, including a majority of the CBF regulon genes, the CBF2 Δ C expressing plants were able to cold acclimate. These results are consistent with the hypothesis that cold acclimation pathways independent of the CBF cold response pathway contribute significantly to cold acclimation in *Arabidopsis*.

This work was funded in part by grants to MFT from the National Science Foundation (DBI 0110124), DOE (DE-FG02-91ER20021) and the Michigan Agricultural Experiment Station.

38. Mutant screen for upstream components of the cold acclimation response in *Arabidopsis thaliana*

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Environmental stresses such as cold and drought significantly impact the capacity of plants to survive throughout many regions of the world and result in significant losses in crop productivity on an annual basis. We are interested in understanding the mechanisms that plants have evolved to survive freezing temperatures with the long-range goal of improving the stress tolerance of important crop species. Toward this end, we are studying plant cold acclimation, the process whereby plants increase in freezing tolerance in response to low non-freezing temperatures. Previous work has shown that C-Repeat Binding Factors (CBF) are transcriptional activators that are induced rapidly upon exposure to low, non-freezing temperatures and that they activate expression of a group of genes, the CBF regulon, that impart freezing and drought tolerance (Jaglo-Ottosen et al, 1998; Gilmour, 1998; Stockinger EJ, Gilmour SJ, Thomashow MF, 1997). One current goal is to understand how the CBF genes are induced by low temperature. Two regions of the CBF2 promoter have been shown to be important for the cold induction of CBF2 (Zarka, 2003). In this project, a genetic approach is being taken to identify trans-acting factors that function through these cis-acting regulatory elements of CBF2 to regulate gene expression in response to low temperature. A mutant screen was conducted using plants containing a cold responsive region of the CBF2 promoter fused to the GUS reporter gene. Mutagenized plants were screened for GUS expression after cold treatment and a single mutant was identified with reduced GUS expression after seven days at 4°C. This phenotype could result from a mutation outside of the designated promoter construct that has an effect on expression of the gene at low temperature. Further analysis of this mutation will determine if this is a mutation in a trans-acting factor involved in regulation of the CBF genes.

39. Knock-out Confirmation and Identification of Differences in Functional Roles for CBF 1, 2 and 3 During Cold Acclimation of *Arabidopsis thaliana*

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A phenomenon known as cold acclimation occurs in some plant species whereby they acquire the ability to resist sub-zero temperatures after being subjected to a period of near freezing conditions (Guy, 1990; Thomashow, 1998). Understanding how this cold acclimation response occurs at the molecular level is a major focus of research in an effort to find ways to increase freezing tolerance in plants. Using *Arabidopsis thaliana* as a model system, previous work has identified the CBF pathway as an important component in the process of cold acclimation (Thomashow, 2001). Six different homologs of CBF can be

found in Arabidopsis and three of these (CBF 1, 2 and 3) are of interest for further study. Each is induced highly in response to low temperature in Arabidopsis (Gilmour et al., 1998; Jaglo-Ottosen et al., 1998; Medina et al., 1999) and homologs can be found in many other plant species. These three CBF genes encode transcriptional activators which are members of the AP2/EREBP family of DNA binding proteins (Riechmann and Meyerowitz, 1998). In Arabidopsis all three are in tandem array on chromosome 4 (Gilmour et al., 1998). Over-expression of CBF 1, 2 and 3 individually has been shown to be sufficient for achieving cold acclimation and has indicated that all three target a similar gene set (Gilmour et al., 2000; Gilmour et al., 2004). However, it is not yet clear whether the roles of each CBF gene are functionally equivalent. The focus of this project is on understanding the contribution that each CBF gene makes to the process of cold acclimation. In order to understand the specificity of each CBF gene we have identified homozygous T-DNA insertion lines in each transcript and are screening these to identify knock-outs at the transcript level. Once knock-outs are confirmed, the expression of selected target genes will be analyzed for each line. Finally electrolyte leakage assays will be used to test the effect that each CBF homolog has on freezing tolerance. The results of this study are expected to contribute to the understanding of the role(s) that the conserved forms of CBF play in freezing tolerance for plants that display the cold acclimation response.

40. Effects on Leaf and Protoplast Protein Levels Resulting From NADP-Malic Enzyme Overexpression in Arabidopsis and Tobacco

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Malate is produced in the cytosol of guard cells and is a component of the counter ion pool regulating stomatal aperture in plants. The guard cells either swell or contract as a result of controlled ion pumping and osmosis. In order to facilitate the process, the malate must be either exported or broken down. Malic enzyme can be used to regulate the exchange of gases and water vapor from the leaf to the atmosphere during transpiration. The NADP-malic enzyme (ME) gene converts malate and NADP to pyruvate, NADPH, and CO₂ through oxidative decarboxylation (Edwards and Andreo 1992; Drincovich *et al.* 2001). Overexpression of this single gene has been linked to decreased stomatal pore size, resulting in decreased water loss (Laporte 2002).

We are determining the size and levels of proteins produced as a result of increased ME expression. We conducted these analyses using Western blots to detect the histidine tagged protein. Lines of transgenic Arabidopsis were produced, having the maize ME gene fused to a 35s promoter and a constitutively expressed C-terminal His tag. A second line was transformed with a KAT2 guard cell specific promoter, driving the expression of a C-terminal histidine tagged maize ME gene (Thakur, Laporte Unpublished). We expect these plants to have heightened ME expression isolated around the stomatal guard cell tissue only.

Crude protein extracts were isolated from wild-type Arabidopsis and tobacco whole leaf tissue as well as the 35s and guard cell specific plants. Guard cell protoplasts were also isolated from the WT and 35s plants and protein extracts were collected.

These samples were run on SDS-Page gels alongside a His ladder and a His protein control, isolated from *E. coli* strain BL21. The purified control has a His tag on both ends, the N-terminus from the pET32 vector and a His tag independently fused to the C-terminus. The molecular weight of the protein is 72-kDa and visible as a single band (Andreo *et al.* 1997). Expression of the maize NADP-ME protein in the transgenic Arabidopsis plants, will be presented, along with corresponding images and related findings.

41. NADP Malic Enzyme Alters Guard Cell Function in *Nicotiana tabacum*

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Plants lose most of their water through transpiration. Stomatal aperture affects the amount of water taken up by plants. Overexpression of NADP malic enzyme has been shown to cause a decrease in stomatal aperture. We hypothesize that excess malic enzyme alters malate concentration in guard cells (Laporte et al. 2002). A gene coding for NADP malic enzyme was isolated and cloned into a binary vector, PMP535. The chloroplast transit peptide was removed. The promoter used in this vector was from the KAT2 gene, which is guard cell specific (Pilot 2001). *Nicotiana tabacum* was transformed using *Agrobacterium tumefaciens*. The T₂ generation of plants was screened for homozygous lines using Finale, an herbicide, supplemented into MS media. The putative transformants were screened with PCR. Enzyme assays were completed to quantify the activity of the enzyme. Preliminary analysis of the transgenic plants will be presented.

42. Testing the Role of *ATG3*, *ATG7*, *ATG8*, and *ATG9* During Autophagy by Performing Mutant Analysis and Complementation of Genes in Plants.

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When plant cells are faced with an inadequate supply of nutrients, they begin to eradicate some of their own internal organelles for the reuse of their components; this process is called autophagy (Wang et al, 2003). The importance of autophagy depends on the organism; if an animal or yeast is starving autophagy will delay the organisms' death. For plants, autophagy allows for better adaptation and response to the changing environment despite the fact that they cannot move. Autophagy is well understood in yeast and all 27 ATG genes essential for autophagy to occur have been characterized (Klionsky et al. 2003). Due to sequence similarity between yeast and *Arabidopsis*, the identification of corresponding ATG genes in *Arabidopsis* has been possible (Bassham et al, 2006).

We are investigating the roles that *ATG3*, *ATG7*, *ATG8*, and *ATG9* play in the process of autophagy in plants (primarily *Arabidopsis*). By using data-mining techniques to find the sequences that were available in the public domain (the sequences were taken from various mammals, plants, and fungi) we designed degenerate primers for Atg3, Atg7, and Atg9. We performed touchdown PCR using multiple primer pairs for each of these *ATG* genes (some primer pairs being more successful than others). We will now be using the successful primer sets to clone the respective *ATG* genes from other plant species (for which information concerning the *ATG* genes is not already available).

We have also transformed *Arabidopsis* plants to overexpress Atg8 (a and e) and Atg9. We are currently screening the seeds of Atg8- and Atg9-overexpressing plants to find homozygous plants that will be used for analysis. The Atg8 and 9 in these plants have been tagged with green or yellow fluorescent protein to provide a tool for visualization of autophagosomes in *Arabidopsis*.

43. Boron Stress and Boron Tissue Distribution in *Arabidopsis thaliana* and *Pelargonium X Hortorum*

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The micronutrient boron is essential for plant growth and development. Deficient or excessive levels of this micronutrient result in the formation of growth defects that reduce yield in crop plants and result in discarding of horticultural plants. To study the responses of plants to altered boron supply, we developed a hydroponic system for *Arabidopsis thaliana* in which a healthy root system can be maintained. We describe this hydroponic system and how it may be easily adapted for screening of nutrient uptake mutants in this model plant species. Using this system, we report the symptoms that develop in *Arabidopsis* in response to excess or deficient levels of boron. *Arabidopsis* plants deprived of boron (0.3 mM) exhibit stunted flower development and reduced silique growth due to the reduced fertility and reduced seed set of the flowers. Plants exposed to excess levels of boron (1 or 3 mM) exhibited a chlorosis of the leaves and also a reduced fecundity. In order to understand the general requirements for boron during plant growth, we determined the levels of boron in different root and aerial tissues over a period of four weeks. Our results will provide a better basis for the comparison of our boron study with various crop plant species (see also Poster by Ying Deng *et al.*, at this meeting). We performed a comparison between the uptake and distribution of boron in *Arabidopsis* and the horticultural species *Pelargonium X hortorum* (cultivars – “Nittany Lion Red” and “Patriot Peach”) which is the leading bedding plant produced in midwest greenhouses. We will report on the comparison of requirements for boron between these two dicot species. Our findings are of general relevance to growers that encounter water supplies with high or low levels of this important micronutrient.

44. Soybean amino acid composition changes during aphid infestation

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Plant response to insect herbivores has been well characterized with respect to leaf-chewing insects. However, phloem-feeding insects, like aphids, do not induce the same set of plant responses generally associated with insect attacks and wounding. Nutritionally, phloem sap provides aphids with limiting amounts of amino acids (aa). Thus, we hypothesized that plant defense to aphid herbivory may include reducing the levels of free aa available in the phloem. On the other hand, aphids could induce metabolic changes in the plants, including changes in aa abundance, to improve the quality of their diet (‘metabolic hijacking’).

To test this hypothesis we analyzed the aa composition of soybean leaves and apical stems from soybean aphid (*Aphis glycines*)-infested and uninfested plants at 1, 2 and 3 weeks post-infestation. In general, aa concentration in aphid-infested plants was lower than that of uninfested plants during the first week of infestation. By the third week the trend had reversed with aa concentration higher in aphid-infested than uninfested plants. This pattern is consistent with an initial plant defense response that is later overcome by the aphid through metabolic hijacking.

To analyze whether changes in aa composition affect aphid performance, we measured the intrinsic rate of growth of *A. glycines* on soybeans grown with and without symbiotic N-fixing bacteria, *Bradyrhizobium japonicum*. Soybean plants grown with *B. japonicum* had a different aa composition than those grown without *B. japonicum*. Aphid performance was better on plants grown with nodules.

Moreover, individual aa that were elevated in nodulating plants coincided with those elevated three weeks after aphid infestation. Our results suggest that the aa composition is an important determinant of host plant quality for *A. glycines* colonization of soybean and that this quality is directly affected by *A. glycines* herbivory

45. Role of jasmonic acid in long-distance defense signaling

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Jasmonic acid (JA) production in response to wounding and herbivore attack results in genome-wide changes in transcription, including increased expression of JA biosynthetic genes. Recent studies with tomato indicate that JA biosynthesis is required for production of the mobile wound signal that initiates systemic defense responses, and further suggest that JA itself may act as this signal. To study the role of *de novo* JA synthesis in the systemic defense response in Arabidopsis, we developed a transgenic system in which JA synthesis can be restricted to specific tissues of the plant. Injury to wild type Arabidopsis leaves by mechanical wounding or insect feeding resulted in JA accumulation in local damaged leaves but not in systemic undamaged leaves. A mutant that is defective in a peroxisomal JA biosynthetic enzyme (OPDA reductase3; OPR3) accumulated about 5% wild-type levels of JA in response to wounding. As a consequence, *opr3* mutant plants were impaired in local and systemic expression of several wound-responsive genes. A transgene encoding a GFP-OPR3 fusion protein expressed from a dexametazone (DEX)-inducible promoter was introduced into the *opr3* mutant. Leaves treated with 30 μ M DEX accumulated GFP-OPR3 mRNA and protein within 6 hr of treatment. DEX-induced expression of GFP-OPR3 complemented the JA deficiency of *opr3* plants, and was largely restricted to the area of DEX application. This transgenic approach for manipulating the spatial pattern of JA synthesis is currently being used to examine the role of local JA production in systemic spread of the wound signal.

46. ABA inhibits PCD during vascular element differentiation

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Tracheary elements undergo programmed cell death (PCD) during differentiation; whereas, the self-digestion that occurs during sieve element development has been called a cell semi-death (van Bel & Knoblauch, 2000). One of the major obstacles to studying sieve element development is the difficulty in finding elements at different stages of differentiation. The number of sieve elements in vascular bundles is low and the process of differentiation lasts less than 24 hours (Sjolund, 1997). PCD in barley aleurone cells can be inhibited by ABA as detected by DNA fragmentation; this inhibition could be reversed by removal of ABA (Wang et al., 1996). Could ABA also be used to inhibit PCD in xylem elements and is the process that occurs in phloem elements during self-digestion similarly affected? Pumpkin seeds were treated with 4 mM ABA for 3 days in the dark at 35°C. After 3 days, the average number of mature vascular elements per bundle was counted. There were fewer mature tracheary elements and sieve elements per bundle after ABA treatment compared to a water control ($p < 0.01$). Removal of ABA followed by 2 days incubation with water was compared to 5 days of water treatment to determine if the inhibition of ABA could be released. The number of mature tracheary elements after 5 days was not significantly different than the elements in the control, but the number of sieve elements was significantly higher ($p < 0.05$). ABA inhibited cell differentiation of vascular elements - PCD in the xylem and self-digestion in the phloem - and this inhibition could be reversed. The similar response of tracheary and sieve elements to the ABA hormone suggests the self-digestion or cell semi-death in the phloem shares similar regulation to and may be a modification of PCD.

47. Indications of lipid signaling pathways in the phloem exudate of *Arabidopsis thaliana* and *Perilla ocymoides*

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The view of the phloem function has evolved from that of a simple assimilate transport to a trafficking system for pathogen response and developmental regulators. It is crucial for the transport of mineral nutrients, plant viruses, virus-induced silencing, defense and resistance against pathogen infection, and signaling of environmental conditions. The phloem contains a multitude of compounds: small molecules, peptides and proteins, nucleic acids, and possibly lipids. Analysis of metabolites (by TLC and GC-MS) in the phloem exudate showed that there are indeed lipids and fatty acid present. They display a different pattern when compared to leaf-extract lipids and also show an unusual distribution of fatty acids. In addition, analysis of proteins by LC-ESI-MS indicates the presence of components of lipid metabolism, transport, and signaling systems in the phloem. The role of these proteins and lipids for plant development will be further examined.

This work is supported in part by MSU-Intramural grant # 05-IRGP-313.

48. Identification of homologs of *FT/TFL1* gene family in apple (*Malus domestica* Borkh.)

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Flowering plants show wide variation in seasonal timing of flowering and number and arrangement of flowers within the inflorescence. In crop plants such as apple (*Malus domestica* Borkh.), breeding objectives include selection for specific flowering-related traits to optimize production. The genetic basis of these flowering-related traits has not been extensively explored outside of the reference plants such as *Arabidopsis*. In *Arabidopsis*, three members (*FT*, *TSF*, and *TFL1*) of the evolutionarily conserved *FT/TFL1* family of transcriptional regulators play key roles in multiple aspects of flowering. The paralogous *FT/TSF* genes are highly expressed in leaf tissues under inductive photoperiods and promote a transmissible flowering signal that is perceived at the shoot apex. *TFL1* is expressed in the shoot apex where it regulates inflorescence architecture. The remaining three members of this gene family, *MFT*, *BFT*, and *ATC*, have not been characterized.

To identify and functionally characterize *FT/TFL*-related genes in apple, we analyzed ~300,000 available ESTs and found that the apple genome encodes for potential orthologs of *FT/TSF*, *TFL1*, *BFT*, and *MFT*. Our analysis of the publicly available EST and microarray data suggest that *BFT* and *MFT*, and potential orthologs have acquired developmental functions unrelated to flowering. Interestingly, the apple genome encodes for two distinct *TFL1*-related genes. We are currently using molecular approaches to determine the function of these genes in apple.

As a complementary approach to explore the genetics of inflorescence architecture in apple we evaluated natural variation in this trait using an extensive *Malus* reference collection. We found that unlike cultivated apples, wild *Malus* genotypes exhibit wide variation in flower numbers per inflorescence, ranging from 2 (*M. baccata*) to 17 (*M. pratti*) and we are exploring the potential role of *TFL-1* homologs as the basis for this variation. We generated a mapping population of >800 individuals in preparation for QTL analysis and fine mapping of genes influencing this trait.

49. Late flowering phenotype of the ribonuclease mutant *rns2-2*: a link between P_i metabolism and flowering?

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Extracellular and vacuolar ribonucleases (secretory RNases) have been well-studied at the enzymatic and structural levels. However, little is known regarding their biological functions. One family of secretory RNases, RNase T2, is particularly widespread. The Arabidopsis genome contains 5 genes belonging to this family: *RNS1-5*. While RNS1, 3, 4 and 5 are extracellular or predicted to be in the apoplast, RNS2 is an intracellular enzyme. Moreover, RNS2 is highly expressed at all developmental stages and in most plant organs. Expression patterns suggest that at least RNS1 and RNS2 could be involved in nutrient recycling.

As a first step toward understanding the biological roles of RNS2, we decided to characterize the subcellular localization of this enzyme. Initial computational analyses suggested that RNS2 has a putative secretion signal peptide in the N-terminus, and an ER-retention signal in the C-terminus. Thus, we designed a CFP-RNS2 construct in which CFP was inserted between the secretion signal and the body of the protein to avoid interrupting any localization signal. After production of transgenic plants, the fluorescent proteins were identified by confocal microscopy. We determined that RNS2 is located in specialized ER structures called ER-bodies. These structures are proposed to be a reservoir of hydrolytic enzymes, and an alternative pathway for vacuolar transport.

We also identified a null *RNS2* mutant, *rns2-2*. Mutant plants did not show an altered phenotype when grown under normal conditions. Because it has been proposed that RNS2 is part of a P_i salvaging mechanism, we decided to test if *rns2-2* plant could grow using RNA as the only source of P. WT and *rns2-2* plants were grown in magenta boxes with P_i-rich medium, or medium with no P_i but with high molecular weight RNA as sole source of phosphate. An *RNS1* mutant line, *rns1-2*, was also included. A moderate flowering phenotype was observed for the *rns2-2* plants in Pi-rich medium. These plants bolted later than WT or *rns1-1* plants. In medium with RNA as the only source of P, this phenotype was exacerbated. WT and *rns1-1* plants showed a slower rate of bolting in this condition, while the *rns2-2* mutant failed to bolt even when the other two lines had already achieve 100 % bolting. Our results indicate that the role of RNS2 is in fact to recycle phosphate from internal pools, even in conditions when P_i is readily available. Moreover, we hypothesize that phosphate homeostasis is important for flowering, and RNS2 is necessary to maintain the availability of phosphate during this process.

50. The Role of Two Ubiquitin-like proteins in Arabidopsis Peroxisome Biogenesis and Function

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Plant peroxisomes are dynamic organelles that play a vital role in plant growth and development. PEX2 is an integral membrane protein, which belongs to the RING family and is a critical component of the peroxisomal assembly and matrix protein import machinery. In plants, PEX2 has been shown to be essential for viability and to be involved in the photomorphogenic response (Hu et al, 2002). Yeast two hybrid approach was employed to identify PEX2 interacting factors and resulted in the retrieval of an Ubiquitin-Like protein, named AtPLIC1. *Arabidopsis* was found to have a highly conserved paralog of this

gene, *AtPLIC2*. *AtPLIC* genes are present in tandem and oriented in head to tail fashion on the chromosome. The *AtPLIC* proteins have an N-terminal Ubiquitin-Like (UBL) domain, a C-terminal Ubiquitin Associated domain (UBA) and four chaperonin binding sites intersticed between them. Both *AtPLICs* interact with the RING domain of *PEX2*. Deletion constructs are being made to delineate the *AtPLIC* domain specific to this interaction. Using Fluorescence microscopy we demonstrate that GFP-fusions of *AtPLIC1* and *AtPLIC2* localize to the peroxisome. T-DNA insertion lines of *AtPLICs* were identified and investigated to determine the functional role of the *AtPLICs*. RNAi lines are currently being analyzed to further elucidate the functions of the *AtPLICs* in *Arabidopsis*. UBL-UBA proteins are believed to function as shuttle factors, relaying ubiquitinated proteins to the proteasome. Drawing analogy from the ERAD (Endoplasmic Reticulum Associated Degradation) model, the presence of RING proteins (putative E3 ligases), E2, AAA-ATPases and UBL-UBA proteins in the peroxisomes prompts us to speculate that peroxisomes also have a Ubiquitin-proteasome type of proteolytic system associated with them, which regulates peroxisome biogenesis and function by targeting selective peroxisome proteins for degradation.

51. Light Regulated Expression of *PEX11b* Gene in Arabidopsis

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Peroxisome division is poorly understood in higher organisms. The *PEX11* peroxisome membrane proteins are the only known factors to promote peroxisome division and multiplication specifically in plants (Orth Travis *et al.*, 2006). A shift from dark to light remarkably induces elongation of the peroxisome and subsequent division and multiplication in *Arabidopsis* seedlings. We determined the condition and stages of *PEX11b* expression in *Arabidopsis*. The expression of *PEX11b* gene is increased dramatically with increase in time points after dark to light shift of *Arabidopsis* seedlings. *PEX11b* is known to be important in peroxisome division among the other members of its family. *PEX11b* promoter was analyzed for the presence of typical light response elements (LRE's) and several of them were found. A minimal region (~250bp) of the *PEX11b* promoter was able to form a DNA: Protein complex. Biochemical and genetic data indicated PHYA and CRY1 photoreceptors to play a significant role in regulation of *PEX11b* gene expression. A careful examination of the downstream components of PHYA signaling pathway revealed PAT1 (Phytochrome A Transduction) and HYH (HY5 Homolog) to be involved in transcriptional activation of *PEX11b*. In the present work we demonstrate *in vitro* the physical interaction of all the three different forms of bacterially expressed HYH protein and the 250bp *PEX11b* promoter fragment, and further establish this interaction and its important role through genetic evidence. HYH is a positive regulator of photomorphogenesis (Holm *et al.*, 2002). Peroxisomes are essential during seed germination and early stages of growth (Hu *et al.*, 2002). Search of other nuclear proteins that regulate *PEX11b* are important, to understand how light exerts its function in peroxisome proliferation.

52. Do plants contain a ZipA functional analog?

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Chloroplast division is mediated by two types of FtsZ, FtsZ1 and FtsZ2, which are homologues of the tubulin-like bacterial division protein FtsZ. In bacteria, FtsZ encircles the division site and is one of the first proteins to appear at the division site. Similar to tubulin, FtsZ binds proteins at its C-terminus and these proteins are required for proper cell division as either loss of the FtsZ C-terminus, or loss of these

proteins impairs cell division. One of these proteins, ZipA, binds a conserved amino acid sequence of the FtsZ C-terminus and this motif is found in plant FtsZ2 proteins but absent in FtsZ1 proteins.

Due to the presence of a conserved ZipA binding motif in plant FtsZ2 proteins, we have been searching for a homologue to bacterial ZipA in plants. Sequence similarity searches have not yielded an obvious candidate, however, Ssz1, has been identified as being structurally similar to ZipA despite having limited sequence similarity. Ssz1 appears to bind to AtFtsZ2, in a yeast two-hybrid assay. In order to see if Ssz1 could possibly be functionally similar to ZipA we tested Ssz1 by complementing it with an *E. coli* temperature sensitive *zipA* mutant. Despite being an FtsZ2 binding protein, Ssz1 does not appear to complement temperature sensitive *zipA* suggesting that Ssz1 and ZipA may not have similar functional roles *in vivo*.

53. The Genetic Role of FtsZ in Plants

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The model plant organism, *Arabidopsis thaliana*, contains nuclear-encoded FtsZ1 and FtsZ2 families that include three proteins, AtFtsZ1-1, AtFtsZ2-1, and AtFtsZ2-2. Plant FtsZ proteins are homologs of bacterial FtsZ, an ancestral tubulin, and are required for plastid and bacterial cell division. FtsZ forms a ring at the midplastid for chloroplast division. Mutations in these genes result in fewer, enlarged chloroplasts. We are interested in the functional differences between these two gene families. To address this idea we are taking a reverse genetic approach. Nuclear genes are interrupted by insertion of transfer-DNA (T-DNA) from a binary transformation vector. T-DNA insertion mutants for each FtsZ gene are being used to generate double and triple mutants. Segregation of these mutants is determined by PCR genotype and by light microscopy phenotype. Double insertion mutants also have a phenotype of fewer, enlarged chloroplasts and an additional dwarfed whole plant phenotype. Immuno-blot analysis demonstrates that *atftsZ1-1* and *atftsZ2-2* have no detectable protein, and *atftsZ2-1* expression is dramatically reduced. Putative segregating F2 triple mutants have apparent developmental defects such as altered leaf morphology and pigmentation. These data indicate chloroplast division is important for whole plant growth and development.

54. Investigating the Multiple Targeting Pathways that Direct Proteins to Various Membranes within the Chloroplasts.

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To ensure the efficient and correct targeting of proteins to their final destination, chloroplasts have evolved numerous internal protein routing systems. Two pathways have been proposed for the targeting of proteins to the inner envelope membrane (IEM): the stop-transfer and conservative sorting pathways. We propose to investigate why thylakoid proteins and some inner envelope proteins are translocated across the IEM, while other proteins are halted (i.e., via the stop transfer pathway) at the IEM. In addition, we propose to investigate the unique problem of how IEM proteins that use the conservative sorting pathway are specifically redirected to the IEM rather than being mistargeted to the thylakoid membrane. Using standard molecular biology techniques to analyze model IEM and thylakoid proteins, we propose to identify targeting determinants that direct these proteins to their final destination. Several critical biophysical features that could serve as a signature targeting sequence will be considered for this analysis: for example, size and hydrophobicity of a transmembrane domain (TMD); charge distribution; prevalence of prolines and serines near or within a TMD. We anticipate that by identifying and comparing the targeting determinants between IEM and thylakoid proteins, we will be able to identify specific targeting signals that are predictive of whether a protein is to be targeted to either the IEM or the thylakoid membrane.

55. Genetic, Biochemical and Physiological Studies Acetyl-CoA Metabolism via Condensation

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Acetyl-CoA is metabolized via one of three mechanisms, carboxylation, acetylation and condensation. Acetoacetyl-CoA thiolase (AACT) catalyzes the condensation of two acetyl-CoA molecules to form acetoacetyl-CoA. The fate of acetoacetyl-CoA depends on the biological context in which it is generated. In the cytosol of plant cells, it is the precursor of mevalonate-derived isoprenoids. In microbes, such as *Rhodospirillum rubrum*, acetoacetyl-CoA is the precursor of the storage polymer polyhydroxybutyrate (PHB). BLASTP analyses have identified two AACT genes in the *Arabidopsis* genome, At5g47720 (*AACT1*) and At5g48230 (*AACT2*). These two genes code for proteins that share 75% sequence identity. Two T-DNA insertion alleles at each AACT gene have been characterized. These characterizations indicate that although both genes are expressed (as evidenced by RT-PCR analysis), mutations in *AACT2* are embryo lethal, whereas null alleles of *AACT1* are viable and show no apparent growth phenotypes. Additional physiological, morphological, ultrastructural and expression characterizations of these mutants will be conducted.

In *R. rubrum*, the AACT enzyme is encoded within the *phaABC* operon, which is responsible for PHB biosynthesis. Furthermore, *R. rubrum* contains two additional AACT-like genes, called *phaC2* and *phaC3*. To characterize the roles of these genes in acetyl-CoA metabolism, we have generated antibodies to each gene product. In addition, we are developing an inducible-expression system for individually over-expressing each *pha* gene. In combination, these studies will elucidate the role of AACT in the acetyl-CoA metabolic network.

56. Regulation of Membrane Lipid Homeostasis by RAO1 in Arabidopsis

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The essential galactolipids mono- and di-galactosyldiacylglycerol (MGDG and DGDG, respectively) are the predominant lipid species found in plant chloroplasts. In the case of DGDG, the major biosynthetic route involves digalactosyldiacylglycerol synthase 1 (DGD1)—as the *dgd1* null mutant in *Arabidopsis* shows a ~90% reduction in steady-state DGDG levels and severely stunted growth. Under P_i-limiting growth conditions, however, a *DGD1* paralogue (*DGD2*) shows marked up-regulation at the transcript level and *DGD2* is part of an “alternative” galactolipid biosynthetic route (along with the P_i-regulated MGD1 paralogues MGD2/3). To date, little is known about the mechanism(s) by which the alternative galactolipid biosynthetic route is regulated in regard to P_i availability. A suppressor screen in a *dgd1* ethane methyl sulfonate mutagenized population recovered a mutant that shows constitutive activation of alternative DGDG biosynthesis and has been mapped to a gene encoding a protein of unknown function with two predicted trans-membrane spanning domains (hereafter referred to as RAO1, Regulator of Alternative Oxidase 1). Recent analyses have revealed that RAO1 is localized to the mitochondrion and that the suppressor “gain of function” allele, *rao1-1*, results in a drastic reduction in the protein levels of alternative oxidase (AOX) while a “loss of function” T-DNA insertion allele, *rao1-2*, shows no reduction in AOX protein levels. Steady-state H₂O₂ levels were found to be elevated in *rao1-1* plants, and exogenously applied H₂O₂ and SHAM (an AOX inhibitor) were shown to activate alternative DGDG biosynthesis in *dgd1* plants. As AOX is a terminal oxidase in the electron transport chain that has been proposed to limit reactive oxygen species (ROS) production in plant mitochondria, these findings implicate a novel, potential role for mitochondrial generated ROS in regulating alternative galactolipid biosynthesis in P_i-deprived plants through a signal transduction pathway involving RAO1.

57. Characterization of Phenotypic Differences Between *Arabidopsis thaliana* Autotetraploids and Diploids

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Polyploidy is the condition where an organism has additional sets of chromosomes beyond the diploid condition. Polyploidization occurs via changes in chromosome numbers in gametes followed by either self or cross fertilization within one species (autopolyploid) or mating between two different species (allopolyploid). Throughout time, plants have undergone one or more whole genome duplications, or polyploidization events. It is thought that the resulting increase in gene number can potentially lead to long term increases in fitness and reproductive success; however, little is known about how polyploid and diploid plants differ phenotypically. In order to determine if there is a characteristic of polyploids that distinguishes them from diploids and potentially confers a fitness advantage, we are characterizing phenotypic differences between *Arabidopsis thaliana* autotetraploid (4n) and diploid (2n) individuals. Specifically, we are comparing the only known naturally occurring *Arabidopsis* autotetraploid ecotypes, Warschau and M7943s, with their closest relatives based on DNA sequence polymorphism data (Nordborg et al., 2005). We have begun comparing traits such as time to flowering, leaf number, rosette diameter, plant height, root growth, biomass, and seed set across the ecotypes. We will also compare physiological traits such as photosynthesis, transpiration and gas exchange. These parameters will then be measured under different environmental conditions to determine if there are differences in response to abiotic and biotic stresses. Together, these experiments will give insight into the distinguishing attributes of polyploids that may impart a fitness advantage or disadvantage compared to diploids.

58. A new lab-based synthetic method for making 2-D-carboxyarabinitol 1-phosphate, a regulatory inhibitor of the Calvin cycle enzyme Rubisco

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Ribulose biphosphate carboxylase/oxygenase (Rubisco) is the primary carboxylating enzyme of the Calvin cycle in photosynthesis. Rubisco activity is regulated under conditions of low light or darkness, one regulatory molecule being 2-D- carboxyarabinitol 1-phosphate (CA1P). CA1P binds tightly to the active site of Rubisco, where it is a competitive inhibitor of carboxylation, similar in structure to the reaction intermediate 2- carboxy 3-ketoarabinitol 1,5-bisphosphate. Glycine max and *Phaseolus vulgaris* are two important crop plants which produce large amounts of CA1P, therefore reducing carbon fixation and potential crop yield under low light conditions. Manipulation of the natural synthesis and degradation pathways of CA1P may lead to increased crop yields. CA1P cannot be purchased, so it must be synthesized in the laboratory. The current synthetic method involves first the addition of labelled cyanide to the carbonyl group of ribulose biphosphate, followed by hydrolysis of the 5- phosphate to form CA1P. A more economical method would involve first hydrolysis of the 5-phosphate from ribulose biphosphate, followed by addition of labelled cyanide at a later step to produce CA1P. Comparison of these two synthetic methods will be discussed.

59. Synthesis and Degradation of 2-Carboxyarabinitol 1-Phosphate and its Regulation of Ribulose Bisphosphate Carboxylase/oxygenase in Soybean Leaves

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2-carboxyarabinitol 1-phosphate (CA1P) acts as a diurnal inhibitor of Ribulose Bisphosphate Carboxylase/oxygenase (Rubisco), the primary enzyme in the Calvin cycle. In soybean and other plants, CA1P accumulates in the leaf at night and binds to the Rubisco enzyme, inhibiting it. As light intensities of the morning increase, CA1P is slowly metabolized allowing Rubisco activity to increase. Plants use various mechanisms to regulate Rubisco in response to light levels. French bean (*Phaseolus vulgaris*), predominantly employs CA1P, while beet (*Beta vulgaris*) and soybean (*Glycine max*), utilize a smaller pool of CA1P and may also depend on activation/deactivation of Rubisco. This study investigated degradation and synthesis of CA1P as it regulates Rubisco. Our data contradicts previous data suggesting that CA1P is synthesized faster in low light than in darkness. A low light to darkness transition in a growth chamber resulted in a greater decline in total Rubisco activity in both *P. vulgaris* and *G. max* leaves than a high light to low light transition. Under the conditions used, CA1P is synthesized in low light ($5 \mu\text{mol photons m}^{-2}\text{s}^{-1}$), yet not as fast as CA1P is synthesized in darkness. *P. vulgaris* leaves synthesize greater amounts of CA1P and is more dependent on CA1P for Rubisco regulation than *G. max* leaves. Our data also show that *G. max* leaves depend almost exclusively on CA1P as means of regulating Rubisco. After an 8-h dark period, soybean and French bean plants were exposed to a 10 minute “preillumination” by relatively high light intensities followed by stepped increases in light levels simulating “dawn” in a growth chamber. Under the conditions used, *G. max* cv. Mukden depended almost exclusively on CA1P to regulate Rubisco and minimally on activation of Rubisco, which remained at 60-70% during the dark to light transitions. Rubisco in *P. vulgaris* leaves exhibited the same characteristics. A predawn illumination consisting of a ten minute high light exposure appeared to be more effective at removing CA1P from Rubisco in *G. max* than in *P. vulgaris* leaves. This is because the low light levels following preillumination allowed considerable resynthesis of CA1P in *P. vulgaris*, but not in *G. max*. Exogenous alkaline phosphatase was tested for its effectiveness in degrading free CA1P in leaf extracts. Our data suggest that the presence of 10 units/ml alkaline phosphatase allows all CA1P to be metabolized in *P. vulgaris* leaves terminating possible artifactual binding of CA1P during homogenization of leaves. The free CA1P is most likely degraded before the CA1P bound to Rubisco. Oxygen evolution was measured to calculate the photosynthetic induction period of *P. vulgaris* leaves with varying lengths of preillumination. Our results show *P. vulgaris* leaves exhibit longer lag periods than *G. max* leaves to reach maximum photosynthetic rates when illuminated after being in the dark. Preillumination has a greater effect on decreasing lag time in *P. vulgaris* leaves than *G. max* leaves suggesting species with greater levels of CA1P experience a greater reversal in Rubisco inhibition and a more rapid increase in carbon fixation. Treatments of 25 mM ammonium sulfate added to the homogenization medium were used to prevent binding of free CA1P to Rubisco in dark samples, and to assess the amounts of this inhibitor that remained unbound to Rubisco *in vivo*. Most CA1P is bound to Rubisco. Knowledge of the synthesis and degradation of CA1P and manipulation of the mechanism of regulation of Rubisco via CA1P may facilitate increased rates of CO₂ fixation during early morning hours. Compounded over the course of a growing season this could allow faster crop growth in soybean.

60. Glycoarray, a Novel Technology for Screening of Plant Cell Wall Glycosyltransferase Activities

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The plant cell wall is a well-organized network of polysaccharides with varying degree of complexity. The diversity in the linkages connecting all monosaccharides within these polymers indicates the need for a diversified set of glycosyltransferases to catalyze these glycosidic linkages. The development of a methodology that allows the investigation of the activity of these glycosyltransferase at any given time is required to determine their potential role in building cell walls. A detailed study of these glycosyltransferase activities will help understand the mechanism of cell wall elaboration as whole. Although microarrays offer the possibility for gene expression and profiling of glycosyltransferase genes, no tool is currently available to study a set of glycosyltransferase activities at the same time. The goal of this study is to develop an easy and efficient way to investigate the activity of several glycosyltransferase activities simultaneously and in a high throughput manner. Glycochip technology has been used mostly to study carbohydrate-protein interactions and carbohydrate-antibody recognition. Using AtXT1 and AtFUT1, two well characterized glycosyltransferases involved in xyloglucan biosynthesis, we optimized [¹⁴C]Xyl and [¹⁴C]Fuc incorporation onto different acceptors. Several acceptors, including oligosaccharides and polysaccharides, were tested at various concentrations. The radiolabel can be detected and quantified using a simple high resolution phosphorimager. We were able to quantify as little as 90cpm [¹⁴C]-radiolabel incorporation on the slides on 1mm square spots. Our data suggest that glycochip technology can be adapted to glycosyltransferase assays and represent a promising methodology for high throughput screening of new glycosyltransferase activities.

61. Using Fenugreek as a Model System to Study Regulation of Galactomannan Biosynthesis

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Plant cell walls are composed mainly of polysaccharides, including cellulose, hemicelluloses (e.g., xyloglucans, mannans, glucuronoarabinoxylans, and mixed linkage glucans), pectin (mainly present in primary walls) and lignin (only present in secondary walls). In addition to functioning as structural macromolecules, wall polysaccharides serve as reserve substances, particularly in seeds, in many plants. Although the structure of plant cell wall components has been well characterized, little is known about the biosynthesis of wall polysaccharides. Only recently have some enzymes involved in cell wall polysaccharide biosynthesis been identified and characterized, and almost nothing is known about the regulation of wall polysaccharide biosynthesis. We are interested in studying the regulation of hemicellulose biosynthesis using developing fenugreek (*Trigonella foenum-graecum* L.) seeds as a model system. Fenugreek is an ideal plant system for such research because its seed accumulates large quantities of a single polysaccharide, galactomannans, as a reserve polysaccharide in the endosperm during seed development. The dynamics of galactomannan accumulation and related mannosyltransferase and galactosyltransferase (GalT) enzymatic activities was previously studied (Edwards *et al.*, 1992, *Planta* 187:67-74). We hypothesize that the expression of genes for enzymes and proteins involved in galactomannan biosynthesis should be coordinately up-regulated in the endosperm of seed during active galactomannan accumulation. To examine this hypothesis, we have conducted Northern blotting to define seed developmental stages by detecting the transcript levels of the genes encoding two galactomannan biosynthetic enzymes, galactomannan mannan synthase (ManS) and GalT. The transcripts for both genes were found in seeds of 30-41 DAA (days after anthesis), with the highest level at around 35 DAA. We intend to construct cDNA libraries from the endosperm of seeds at developmental stages with active *ManS*

and *Galt* transcript accumulation. We will use EST sequencing to identify candidates for other genes involved in galactomannan biosynthesis and eventually to identify factors that regulate the coordinate expression of these galactomannan biosynthetic genes.

62. Biochemical Characterization and Cloning of Putative Fucosyltransferases for AGPs

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Arabinogalactan-proteins (AGPs) are hyperglycosylated members of the hydroxyproline-rich glycoprotein family (HRGPs). AGPs are found in the cell walls, plasma membranes and extracellular secretions of plants. Due to the complexity of AGPs, we anticipate that 16 enzyme activities are required for synthesis of their arabinogalactan polysaccharide moiety in *Arabidopsis*. None of these enzymes have been cloned. *Arabidopsis* AGPs contain α -1,2-linked fucose residues. We have identified and characterized 9 *Arabidopsis* genes homologous to the xyloglucan- α -1,2-fucosyltransferase gene (*AtFUT1*) that fucosylates xyloglucans (Sarria et al., 2001 *Plant Phys.* 127:1595-1606). These 10 *Arabidopsis* fucosyltransferases (FUTs) are grouped in family GT37 according to the Carbohydrate-Active enZymes (CAZy) database. Among these enzymes, only *AtFUT1*'s function is known. Based on our preliminary data, we hypothesize that *AtFUT4* (*At2g15390*) and *AtFUT6* (*At1g14080*) encode enzymes that are AGP-specific FUTs. Work on the *mur1* mutant showed that the fucose residues of AGPs are important in root cell elongation (van Hengel, Roberts, 2002 *Plant J.* 32:105-113). The predicted amino acid sequences for *AtFUT4* and *AtFUT6* show 57.2% and 61.8% similarity with *AtFUT1* respectively, and share three conserved motifs with *AtFUT1* and known α -1,2 or α -1,6 FUTs from humans, nematodes and bacteria. Bioinformatic analysis predicted *AtFUT4* and *AtFUT6* to be type II membrane proteins consistent with a Golgi localization. We cloned both genes and made his-tagged and untagged constructs for heterologous expression in *Drosophila* S2 and *Pichia* cells. A biochemical assay will be optimized using microsomes from roots and defucosylated AGPs. The assays will be used to evaluate the biochemical function of these putative AGP-FUTs.

63. Efficient Production of Microbial Cellulase Within Recombinant Maize Biomass Converts AFEX-Pretreated Corn Stover into Fermentable Sugars for Alcohol Fuels

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Commercial conversion of lignocellulosic biomass to fermentable sugars requires inexpensive bulk production of biologically active cellulase enzymes, which might be achieved through direct production of these enzymes within the biomass crops. Transgenic corn plants containing the catalytic domain of *Acidothermus cellulolyticus* E1 endo-1,4- β glucanase (*E1*) and the *bar* Bialaphos resistance coding sequences were generated after Biolistic® bombardment of immature embryo-derived cells. *E1* sequences were regulated under the control of the Cauliflower Mosaic Virus (CaMV) 35S promoter and Tobacco Mosaic Virus (TMV) translational enhancer, and *E1* protein was targeted to the apoplast using the signal peptide of tobacco pathogenesis-related protein (Pr1a) to achieve accumulation of this enzyme. The integration, expression and segregation of *E1* and *bar* transgenes were demonstrated respectively via Southern and Western blotting, and progeny analyses. Accumulation of up to 1.13% of transgenic plant total soluble proteins was detected as biologically active *E1* by enzymatic activity assay. The corn-produced heterologous *E1* could successfully convert Ammonia Fiber Explosion (AFEX)-pretreated corn stover polysaccharides into glucose as a fermentable sugar for ethanol production, confirming that the *E1* enzyme is produced in its active form.

64. Enhanced conversion of plant biomass into glucose using transgenic rice-produced endoglucanase for cellulosic ethanol.

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The catalytic domain of *Acidothermus cellulolyticus* thermostable endoglucanase gene (encoding for endo-1,4-b-glucanase enzyme or E1) was constitutively expressed in rice. Molecular analyses of T1 plants confirmed presence and expression of the transgene. The amount of E1 enzyme accounted for up to 4.9% of the plant total soluble proteins, and its accumulation had no apparent deleterious effects on plant growth and development. Approximately 22 and 30% of the cellulose of the Ammonia Fiber Explosion (AFEX)-pretreated rice and maize biomass respectively was converted into glucose using rice E1 heterologous enzyme. As rice is the major food crop of the world with minimal use for its straw, our results suggest a successful strategy for producing biologically active hydrolysis enzymes in rice to help generate alcohol fuel, by substituting the wasteful and polluting practice of rice straw burning with an environmentally friendly technology.

65. Expanding Cloning Possibilities and Vectors Assembly using Zinc Finger Nuclease

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Cloning vectors, which have been specifically designed to facilitate the expression of foreign genes in plants cells, are available from various sources. Nevertheless, the basic design of most of these vectors limits them to the cloning of a single target gene, typically under a specific promoter, in parallel with the expression of a selection gene from the same vector. A vector cloning system was recently developed in which multiple expression cassettes, cloned in small satellite plasmids, are assembled into a binary plasmid using a set of rare-cutting enzymes, allowing the construction of up to seven expression cassettes in a single *Agrobacterium* binary vector. To overcome the problem of the very small number of commercially available rare-cutting enzymes, a set of zinc finger nucleases (ZFNs) and compatible plasmids were developed. ZFNs are artificial restriction enzymes which can be custom-designed to recognize and cleave specific 24- to 30-bp long sequences. These enzymes are composed of a custom-designed DNA-binding domain fused to the *FokI* endonuclease. A set of different ZFNs were produced by *de-novo* assembly of their DNA-binding regions from overlapping oligos containing modified helices responsible for DNA triplet recognition, and they were cloned into bacterial expression plasmids. A compatible set of satellite plasmids was also developed, in which each expression cassette was flanked by recognition sites of different ZFNs. Following their expression in *E.coli*, ZFNs were used to cleave their corresponding expression cassettes from the satellite plasmids, and these cassettes were then cloned into a single binary plasmid. The ability to specifically design, construct, express and use new artificial ZFNs for cloning purposes opens the way for the assembly of multiple expression cassettes for plant genetic engineering.

67. Leaf Fatty Acid Composition in Wheat Lines with Rigid Pubescence

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Rigidly pubescent leaves reveal oil inclusions at distal stretches of trichomes, whereas similar oil bodies of softly pubescent hair cells remain distributed along the trichomes. Leaves are extracted with chloroform:methanol (2:1, v/v). After methylation at 60-70°C for 30 min leaf material is repeatedly extracted with hexane and loaded onto a Cellite-545 column equilibrated with 20% PEGA (polyethylene glycol adipate) for standard gas-liquid chromatography (Kates, 1972) at 188-230°C for 1 hr. Fatty acid profile for leaves with rigid pubescence at the stage of seedlings shows a 20% greater content of C18:3 (linolenic acid, LA) moiety in comparison to similar profile for softly pubescent leaves. A 15.6-23.3% increase of C18:3 moiety to 18:1 and 18:2 fatty acids in rigidly haired leaves is detected when compared with softly pubescent leaves. Higher quantities for other simple lipids (C14:0, C15:0, C16:0, C18:0, and C20:1) are also shown for rigidly pubescent leaves, except C18:1 and C18:2 classes displaying 5.3-5.9% more abundance in softly pubescent leaves. Prevention of lipid 'solidification' by higher cellular concentrations of LA may account for intactness of rigid trichomes observed throughout wheat ontogenesis, and providing higher buoyancy of integrated membrane proteins.

68. Regulation of Stomatal Density by NADP-Dependent Malic Enzyme in *Arabidopsis thaliana*

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Plants lose majority of their water as transpiration due to stomatal opening and closing, which is governed by guard cells surrounding the stomata. Ions such as K^+ and Cl^- enter into the guard cells from neighboring cells, while malate is synthesized within the guard cells. Water passively follows these ions into the guard cells and their turgidity increases which leads to opening of the stomata (Salisbury and Ross 1994). Stomata close when K^+ and Cl^- are transported out, and the cytosolic isoform of NADP malic enzyme converts malate to pyruvate (Outlaw et al., 1981). This study will examine a possible mode for regulating stomatal density by over-expressing NADP-dependent malic enzyme (NADP-ME) in *Arabidopsis* guard cells. Over expression of C₄ ME in tobacco using a constitutive promoter resulted in a decrease in the stomatal aperture. However, it was not possible to conclude that it was the ME activity in guard cells specifically that was responsible for decreased stomatal aperture (Laporte et al., 2002). We have developed transgenic *Arabidopsis* plants that are transformed with a guard cell specific promoter driving expression of maize NADP malic enzyme. Enzyme assays show higher ME activity in these transformants, indicating an active form of maize ME. We are in the process of understanding the effects of NADP-ME over-expression in *Arabidopsis*. First, scanning electron microscopy was employed to determine if any differences exist in numbers of stomatal complexes among transgenic lines and wild-type *Arabidopsis*. Second, we utilized a native PAGE assay to confirm NADP-ME over-expression occurs only in guard cells. The results indicate a significant increase in stomatal numbers among the transgenic plant lines relative to the wild-type. Altering NADP-ME expression levels may present a novel way for influencing stomatal density which could control plant water loss through transpiration.

69. pSAT Vectors: A Modular Series of Plasmids for Assembly of Multiple Gene Expression, Epitope Tagging and RNAi

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Today, cloning vectors that have been specifically designed to facilitate the fusion, over-expression or down-regulation of a variety of genes in plant cells are available from various sources. In most cases, their basic design allows the cloning of only a single target gene, typically under a specific promoter, in parallel with the expression of selection and/or marker genes from the same vector. Thus, most cloning systems suffer from a limited number of tags, selection markers, promoters and terminators restricting the

expression of multiple target genes on a single plasmid. We therefore developed a new modular satellite (SAT) vector system that supports (i) N- and C-terminal fusions to six different autofluorescent tags, EGFP, EYFP, Citrine-YFP, ECFP, RFP, and DsRed2, (ii) expression of the target genes under the control of the 35S, ocs, nos, mas, act, and rbc constitutive promoters, (iii) RNAi-mediated gene silencing and (iv) N- and C-terminal fusions to various peptide epitopes. All these vectors carry an expanded multiple cloning site that allows an easy exchange of the target genes among autofluorescence, BiFC, and epitope tags. Furthermore, individual expression cassettes can be assembled into *Agrobacterium* binary plasmids, allowing an efficient transient and stable expression of multiple, free and tagged proteins following a biolistic delivery of the DNA or *Agrobacterium*-mediated genetic transformation.